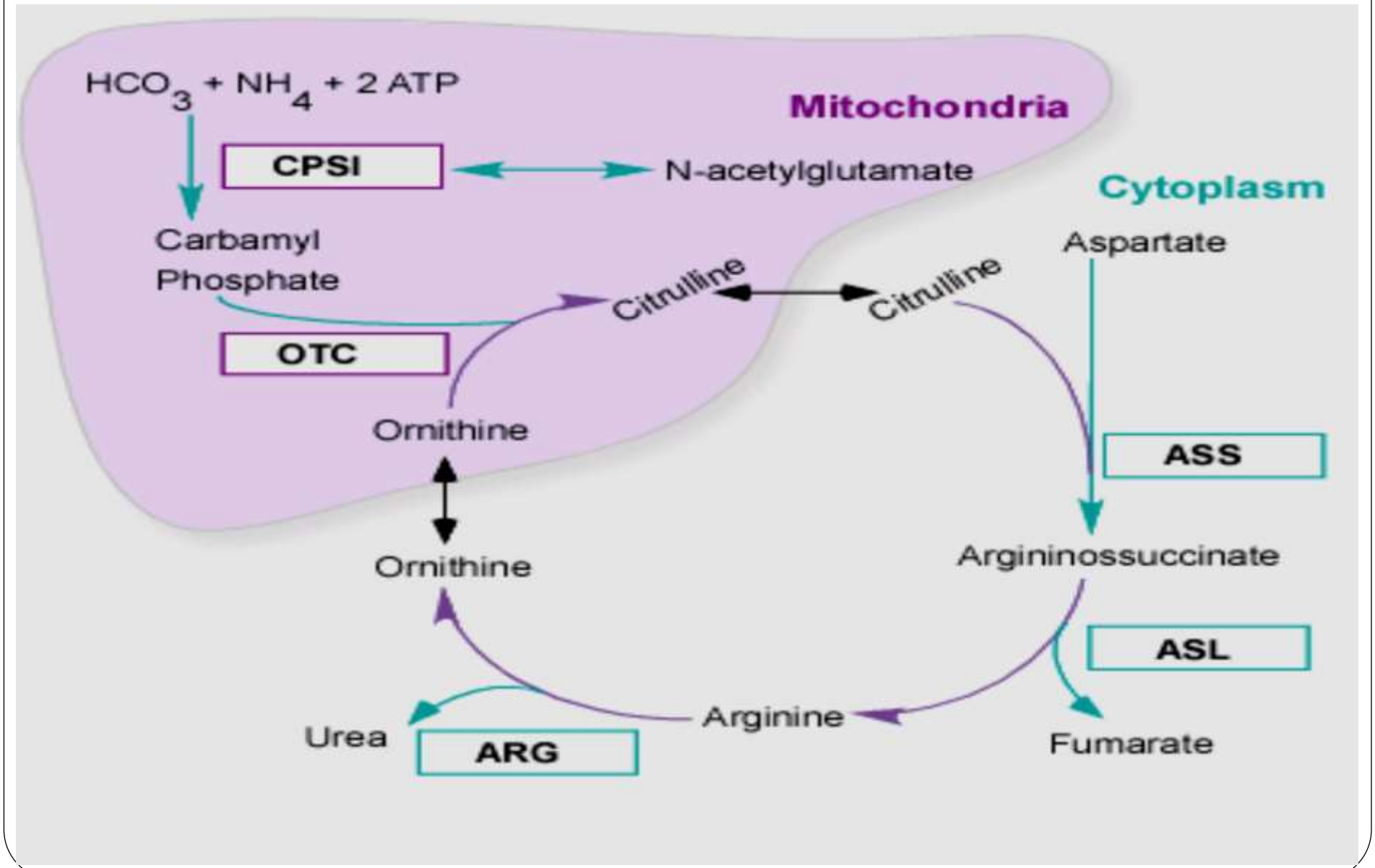
Urea Cycle Disorders

1st lecture in Clinical chemistry

Disease characteristics

- The urea cycle disorders (UCD) result from defects in the metabolism of the extra nitrogen produced by the breakdown of protein and other nitrogen containing molecules.
- Severity of the disease is influenced by the position of the defective enzyme in the pathway and the severity of the enzyme defect. The catabolism normally present in the newborn period combines with the immaturity of the neonatal liver to accentuate defects in these enzymes.

Urea Cycle


HCO_3 + NH_4 + 2 ATP
Mitochondria
CPSI
N-acetylglutamate
Cytoplasm
Carbamyl Phosphate
Aspartate
Citrulline
Citrulline
OTC
Ornithine
ASS
Ornithine
Argininosuccinate
ASL
Urea
ARG
Arginine
Fumarate

Clinical Manifestations

- The urea cycle disorders (UCD) result from defects in the metabolism of the extra nitrogen produced by the breakdown of protein and other nitrogen containing molecules. This extra nitrogen is converted into ammonia (NH4) and transported to the liver where it is processed via the urea cycle, composed of five enzymes in the direct pathway and one enzyme, NAGS, which makes a necessary cofactor .

The enzymes in order in the pathway are:

1: Carbamyl phosphate synthase I **(CPSI)-----*CPSI Deficiency***

2: Ornithine transcarbamylase **(OTC)----*(OTC Deficiency)***

3: Argininosuccinic acid synthetase **(ASS)----*Citrullinemia:*** *(ASS Deficiency)*

4: Argininosuccinic acid lyase **(ASL)---*Argininosuccinicaciduria:*** *(ASL Deficiency)*

5: Arginase **(ARG)---*Argininemia*** *(ARG Deficiency)*

Co-factor: N-acetyl glutamate synthetase **(NAGS)---*(NAGS Deficiency)***

Signs & symptoms

- Infants with a urea cycle disorder often initially appear normal but rapidly develop cerebral edema and the related signs of lethargy; anorexia; hyperventilation or hypoventilation; hypothermia; seizures; neurologic posturing; and coma.
- In milder (or partial) urea cycle enzyme deficiencies, ammonia accumulation may be triggered by illness or stress at almost any time of life, resulting in multiple mild elevations of plasma ammonia concentration. The hyperammonemia is less severe and the symptoms more subtle.
- In patients with partial enzyme deficiencies, the first recognized clinical episode may be delayed for months or years.

Signs & symptoms

- Because newborns are usually discharged from the hospital within 1-2 days after birth, the symptoms of a urea cycle disorder are often not seen until the child is at home and may not be recognized in a timely manner by the family and primary care physician. The typical initial symptoms of a child with hyperammonemia are non-specific:

  failure to feed, loss of thermo-regulation with a low core temperature, and somnolence.

- **Symptoms** progress from somnolence to lethargy and coma. Abnormal posturing and encephalopathy are often related to the degree of CNS swelling and pressure upon the brain stem. About 50% of neonates with severe hyperammonemia have seizures.

  Hyperventilation, secondary to cerebral edema, is a common early finding in a hyperammonemic attack, which causes a respiratory alkalosis.

  Hypoventilation and respiratory arrest follow as pressure increases on the brain stem.

The mainstays of treatment are:

1) reducing plasma ammonia concentration,
2) pharmacologic management to allow alternative pathway excretion of excess nitrogen,
3) reducing the amount of nitrogen in the diet,
4) reducing catabolism through the introduction of calories supplied by carbohydrates and fat, and
5) reducing the risk of neurologic damage

Diagnosis

- A plasma ammonia concentration of 150 mmol /L or higher, associated with a normal anion gap and a normal serum glucose concentration, is a **strong indication for the presence of a UCD.**
- Plasma quantitative **amino acid analysis** can be used to diagnose a specific urea cycle disorder.
- Plasma **concentration of arginine** may be **reduced** in all urea cycle disorders, ***except ARG*** deficiency, in which it is elevated 5-7 fold.
- Plasma **concentration of citrulline** helps discriminate between the ***proximal and distal UCD***, as citrulline is the product of the **proximal enzymes** (OTC and CPSI) and a substrate for the **distal enzymes** (ASS, ASL, ARG).
- **Urinary orotic acid** is measured to distinguish ***CPSI deficiency and NAGS*** deficiency from OTC deficiency.
- A definitive diagnosis of (CPSI, OTC or NAGS) deficiencies depends on determination of enzyme activity from a **liver biopsy specimen**; however, the combination of family history, clinical presentation, amino acid and orotic acid testing, and,
- in some cases, **molecular genetic testing** are often sufficient for diagnostic confirmation, eliminating the risks of liver biopsy.

Genetic counseling

- Deficiencies of **CPSI, ASS, ASL, NAGS, and ARG** are inherited in an **autosomal recessive** manner.
- **OTC deficiency** is inherited in an **X -linked manner**.
- Prenatal testing for **CPSI deficiency** is available by linkage analysis.
- Prenatal testing for **OTC deficiency** is available by either linkage analysis or direct mutation detection.
- Prenatal testing for **ASS,ASL and ARG deficiencies** is available by biochemical analysis of fetal cells obtained by **amniocentesis** at 16-18 weeks' gestation or by **chorionic villus sampling (CVS)** at about 10-12 weeks' gestation

- Deficiency of any of the **first four enzymes** (CPSI, OTC, ASS, ASL) in the urea cycle or the **cofactor producer** (NAGS) results in the accumulation of ammonia and other precursor metabolites during the first few days of life.

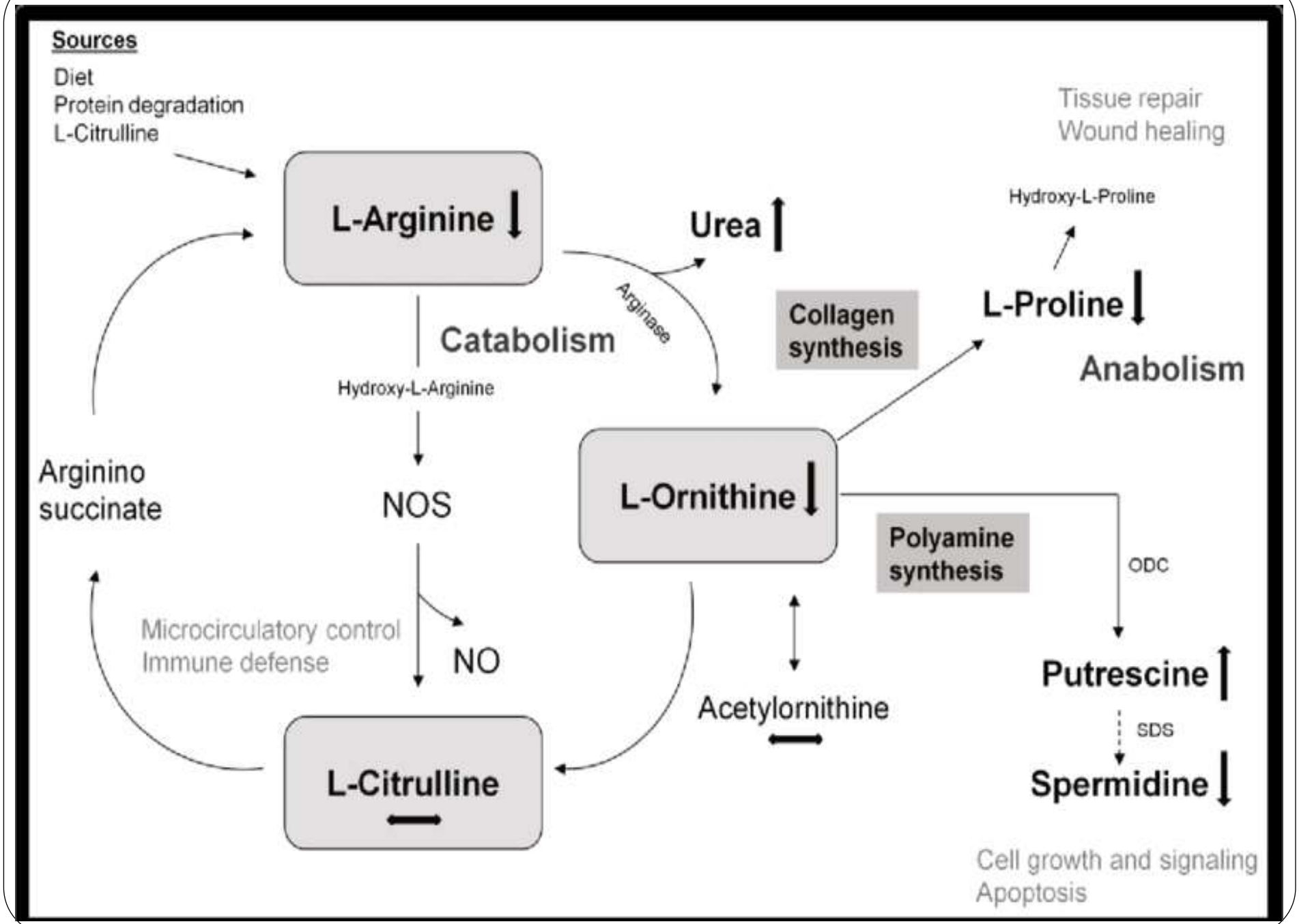
  Since no effective secondary clearance system for ammonia exists, disruption of this pathway results in the rapid development of symptoms
- Deficiency of the fifth enzyme, **arginase**, results in a chronic debilitating disease affecting primarily the nervous system???????.

- In milder (or partial) urea cycle enzyme deficiencies, ammonia accumulation may be triggered by illness or stress at almost any time of life, resulting in multiple mild elevations of plasma ammonia concentration.

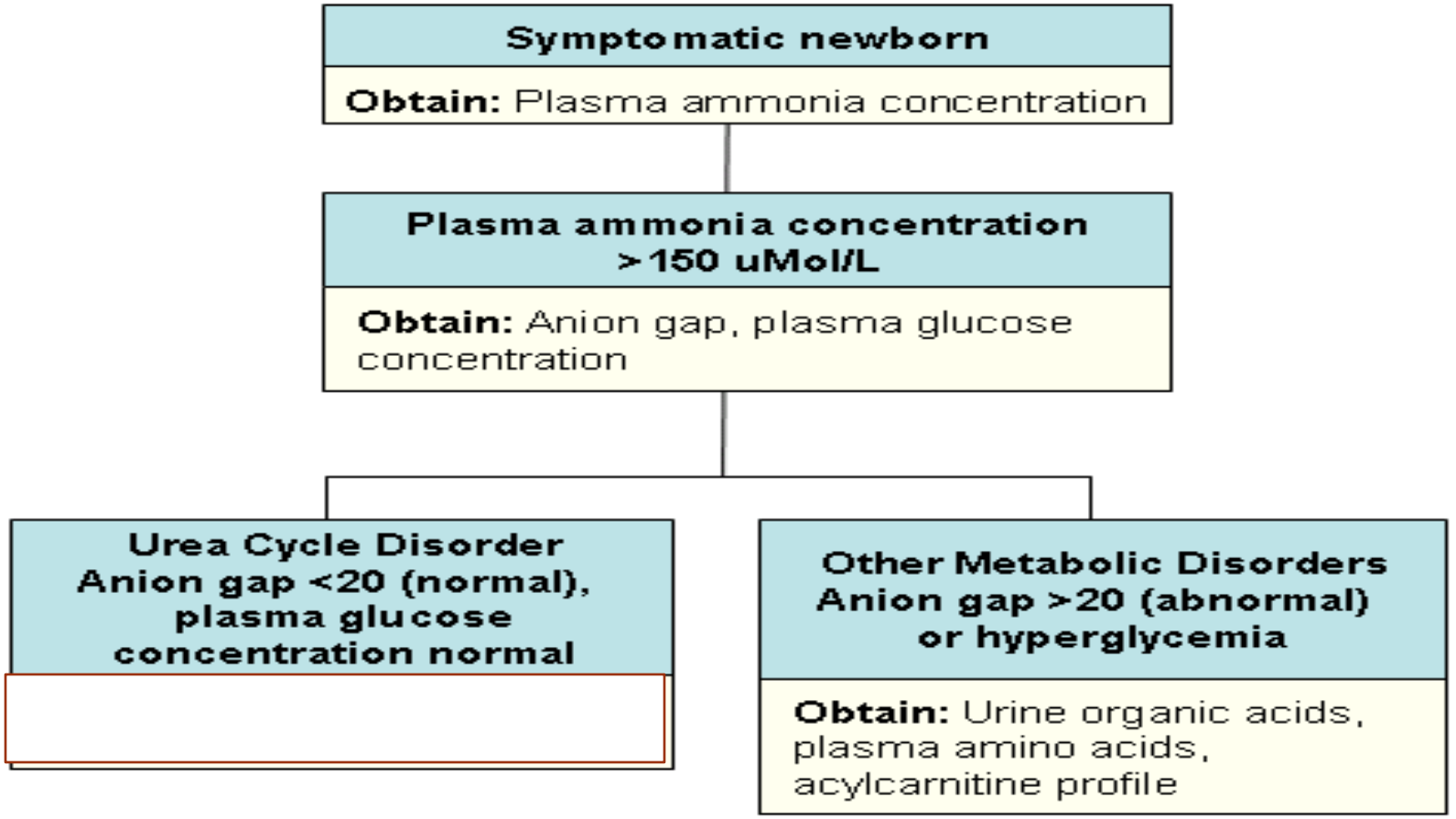
  The hyperammonemia is **less severe** and the symptoms **more faint**.

- In patients with partial enzyme deficiencies, the first recognized clinical episode may be delayed for months or years. Although the clinical abnormalities vary somewhat with the specific urea cycle disorder, in most the hyperammonemic episode is marked by loss of appetite, cyclical vomiting, lethargy, and behavioral abnormalities.

Sleep disorders, delusions, hallucinations, and psychosis may occur. An encephalopathic (slow wave) EEG pattern may be observed during hyperammonemia and non-specific brain atrophy may be seen subsequently on MRI.


Sources
Diet
Protein degradation
L-Citrulline
L-Arginine
Urea
Arginase
Tissue repair
Wound healing
Hydroxy-L-Proline
L-Proline
Collagen synthesis
Anabolism
Catabolism
Hydroxy-L-Arginine
Arginino succinate
NOS
L-Ornithine
Polyamine synthesis
ODC
Microcirculatory control
Immune defense
NO
Acetylornithine
Putrescine
SDS
Spermidine
L-Citrulline
Cell growth and signaling
Apoptosis

Evaluation of a newborn with hyper ammonemia



Causes

- **CPSI deficiency:** Along with OTC deficiency, deficiency of CPSI is the **most severe** of the UCDs. Patients with complete CPSI deficiency rapidly develop hyperammonemia in the newborn period.

- **OTC deficiency:** Absence of its activity in males is as severe as CPSI deficiency. Approximately 15% of carrier females develop hyperammonemia during their lifetime

Causes

- **Citrullinemia (ASS deficiency).** The hyperammonemia in this disorder is quite severe. These patients are able to incorporate some waste nitrogen into urea cycle intermediates, which makes treatment slightly easier.
- **Argininosuccinic aciduria (ASL deficiency).** This disorder also presents with rapid-onset hyperammonemia in the newborn period. This enzyme defect is past the point in the metabolic pathway at which all the waste nitrogen has been incorporated into the cycle.

Treatment requires supplementation of arginine. This disorder is marked by chronic hepatic enlargement and elevation of transaminases. Biopsy of the liver shows enlarged hepatocytes, which may over time progress to fibrosis.

Causes

- **Argininemia (ARG deficiency).** This disorder **is not** typically characterized by rapid-onset hyperammonemia. These patients develop progressive spasticity. They can also develop tremor, ataxia, and choreoathetosis. Growth is also affected.
- **NAGS deficiency.** Deficiency of this enzyme has been described in a number of patients. Symptoms mimic those of CPSI deficiency, since CPSI is rendered inactive in the absence of NAG. Mutation in the gene *NAGS* encoding the protein N-acetyl glutamate synthetase are causative.

Table 1. Molecular Genetics of Urea Cycle Disorders

Disease Name	Gene	Locus	Protein	Molecular Genetic Test Availability
Carbamoylphosphate synthetase I deficiency	*CPS1*	2q35	Carbamoyl-phosphate synthase ammonia	Clinical Testing
Ornithine transcarboxylase deficiency	*OTC*	Xp21.1	Ornithine carbamoyltransferase	Clinical Testing
Citrullinemia	*ASS*	9q34	Argininosuccinate synthase	Clinical Testing
Argininosuccinicaciduria	*ASL*	7cen-q11.2	Argininosuccinate lyase	Research
Argininemia	*ARG1*	6q23	Arginase 1	
NAGS deficiency	*NAGS*	17q21.3	N-acetyl glutamate synthetase	

Evaluation Strategy

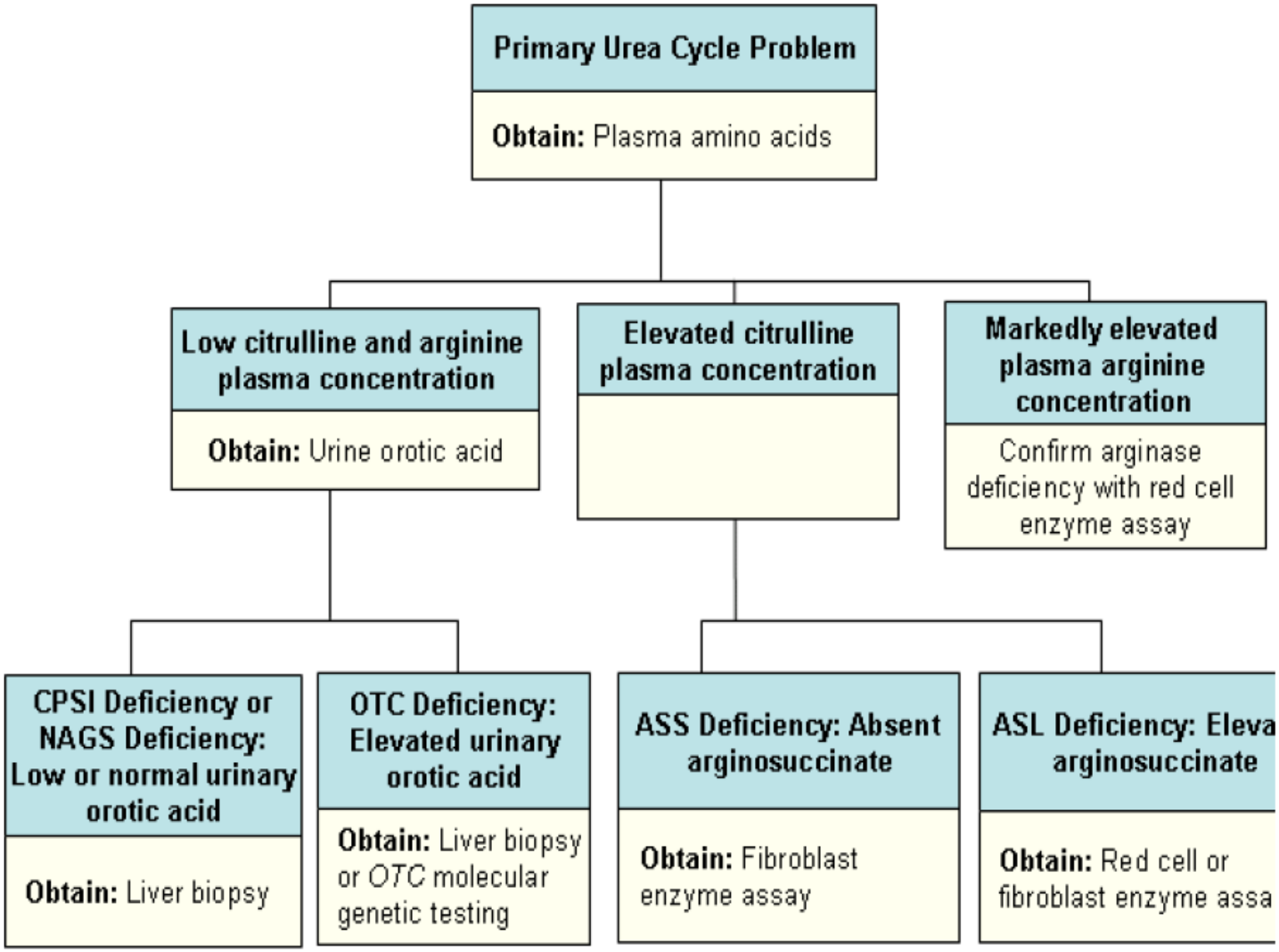
- **Family History**

  A three-generation family history with attention to other relatives (particularly children) with neurologic signs and symptoms suggestive of UCD should be obtained. Documentation of relevant findings in relatives can be accomplished either through direct examination of those individuals or review of their medical records including the results of biochemical testing, DNA - based testing, and autopsy examination. A family history consistent with X-linked inheritance suggests OTC deficiency.

- **Physical Examination**

  No findings on physical examination distinguish among the six types of urea cycle defects; however, **trichorrhexis nodesa** can be suggestive of ASL deficiency.

Test used in the diagnosis of UCD



Plasma quantitative amino acid analysis

- **Plasma concentrations of glutamine, alanine and asparagine** which serve as storage forms of waste nitrogen, are frequently elevated.
- **Plasma concentration of arginine**
- **Plasma concentration of citrulline;** Plasma concentration of citrulline is **markedly elevated** in **citrullinemia and argininosuccinic acidemia**.

Plasma quantitative amino acid analysis

- Patients with citrullinemia have up to a 100-fold elevation in plasma citrulline concentration
- (ASL deficiency) show a more moderate increase in plasma citrulline concentration of about 10-fold, associated with large amounts of argininosuccinic acid, which normally is absent.

Plasma quantitative amino acid analysis

- **Urinary orotic acid is measured** to distinguish CPSI from OTC deficiency. It is significantly **elevated in OTC deficiency** and **normal or low in CPSI deficiency.**

Urinary orotic acid excretion can also be increased in **argininemia** (ARG deficiency) and **citrullinemia** (ASS deficiency) .

- **The argininosuccinate chromatographic peak** may co-elute with leucine or isoleucine, resulting in an apparent increase in one of these amino acids, but its anhydrides eluting later in the run should allow the correct identification of argininosuccinate.

Deficiencies of ASS, ASL, and ARG can be diagnosed on the basis of the amino acid pattern.

Enzyme activity

- Although a definitive diagnosis of CPSI deficiency, OTC deficiency, or NAGS deficiency depends on determination of enzyme activity from a liver biopsy specimen, often the combination of family history, clinical presentation, amino acid and orotic acid testing, and in some cases, molecular genetic testing are often sufficient for diagnostic confirmation, eliminating the risks of liver biopsy.

Molecular genetic testing

- **CPSI Deficiency.**

  The markers used for CPSI deficiency linkage are highly informative and very tightly linked (or intragenic) to the CPS1 locus; thus, they can be used in more than 95% of families with greater than 98% accuracy.

- **OTC Deficiency.**

  75-80% of patients have an *OTC* mutation, identifiable by mutation scanning.

- **Citrullinemia.**

  Linkage analysis is used for carrier detection and prenatal diagnosis.

Disorders of amino acid metabolism

2nd lecture in clinical chemistry 2019

Inborn Errors of Metabolism (IEM)

- IEM comprise a group of disorders in which a **single gene defect** causes a clinically significant block in a metabolic pathway resulting either in accumulation of substrate behind the block or deficiency of the product.
- All IEMs are all **genetically transmitted** typically in an **autosomal recessive** or **X-linked recessive fashion.**

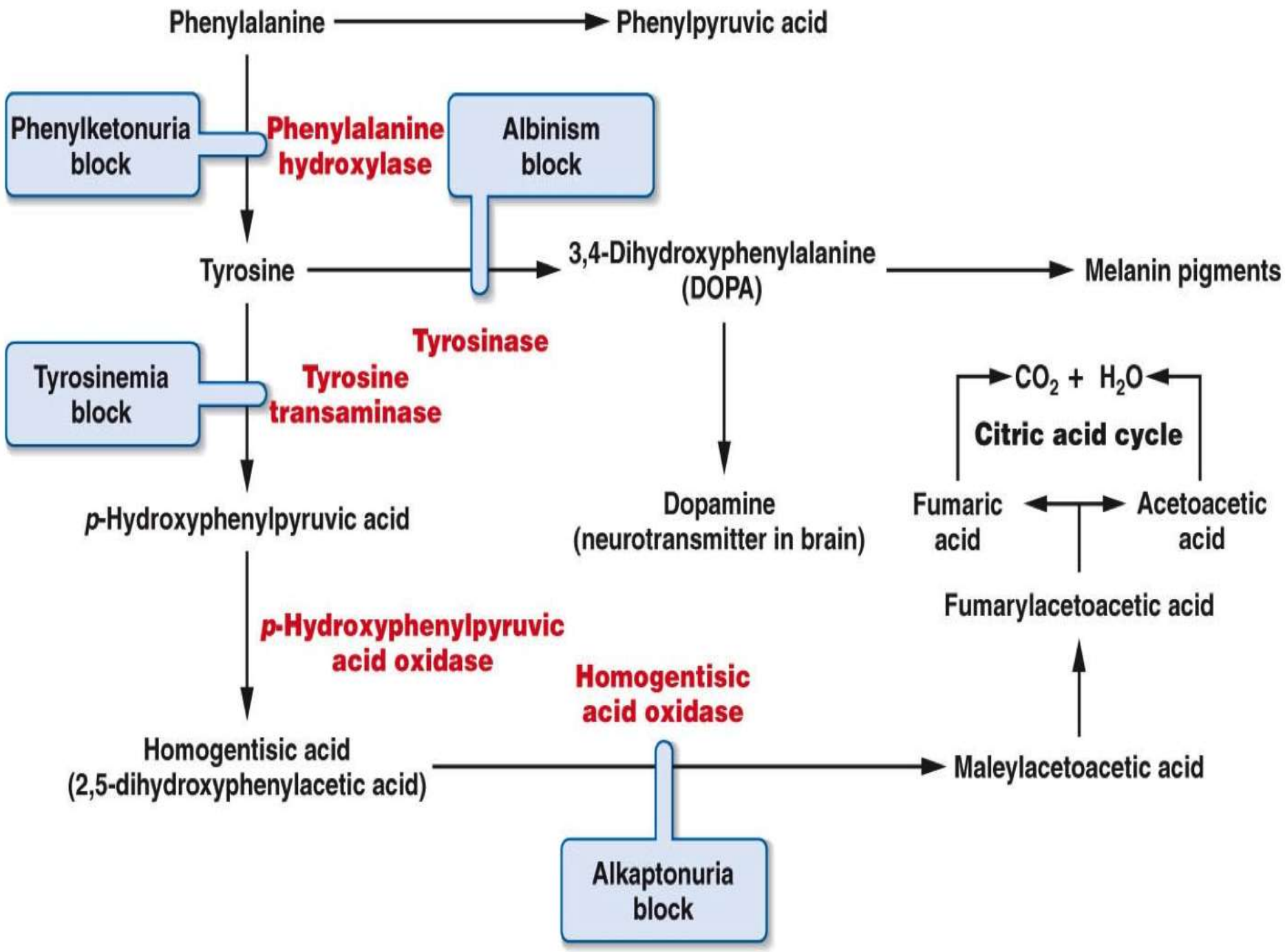
The major types are:

- **Aminoacidopathies** (phenylketonuria, hereditary tyrosinemia, nonketotic hyperglycinemia, maple syrup urine disease [MSUD] and homocystinuria) may have similar presentation to the **organic acidemias**, but are a very heterogeneous group of disorders.
- **Hereditary tyrosinemia** can present in the neonate with a bleeding diathesis due to liver disease, or later in infancy with a renal Fanconi syndrome.
- The severe form of **non ketotic hyperglycinemia** presents as unremitting seizures with hypotonia and hiccoughs.
- **MSUD** classically presents at the end of the first week of life with feeding difficulties, lethargy, coma, seizures and the characteristic odor.

Phenylketonuria PKU

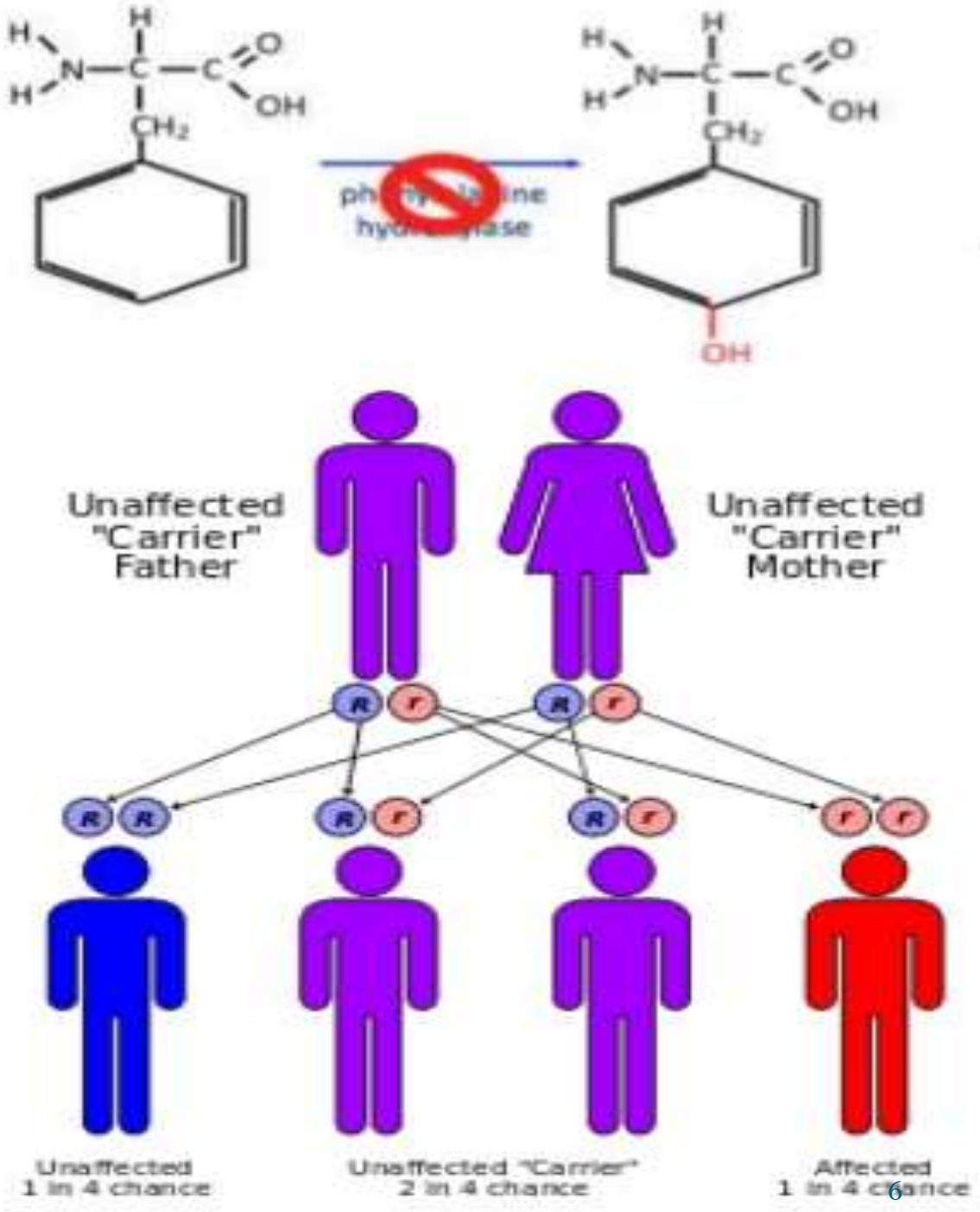
- is an autosomal recessive disorder caused by an abnormality of the **phenylalanine hydroxylase** system.
- In the UK the incidence is about 1 in 10 000.
- Incidence 10 / 100,000 live births in Bahrain, 8/100,000 in Qatar, and 5/100,000 in the UAE. In comparison, the rate of PKU in the US is about 4-6/100,000. Oman 2.4/ 100,000 live births. There have also been documented cases in Algeria, Egypt, Kuwait, and Yemen

- This is the enzyme most commonly affected, but in about 3 % of cases the enzymes responsible for the synthesis of the **cofactor tetra hydro biopterin** are abnormal.
- This means a person must have two faulty copy of PAH genes, which control the PAH enzyme, in order to develop PKU.


Phenylalanine
Phenylpyruvic acid
Phenylketonuria block
Phenylalanine hydroxylase
Albinism block
Tyrosine
3,4-Dihydroxyphenylalanine (DOPA)
Melanin pigments
Tyrosinase
Tyrosinemia block
Tyrosine transaminase
$CO_2 + H_2O$
Citric acid cycle
p-Hydroxyphenylpyruvic acid
Dopamine (neurotransmitter in brain)
Fumaric acid
Acetoacetic acid
Fumarylacetoacetic acid
p-Hydroxyphenylpyruvic acid oxidase
Homogentisic acid oxidase
Homogentisic acid (2,5-dihydroxyphenylacetic acid)
Maleylacetoacetic acid
Alkaptonuria block

Phenylketonuria

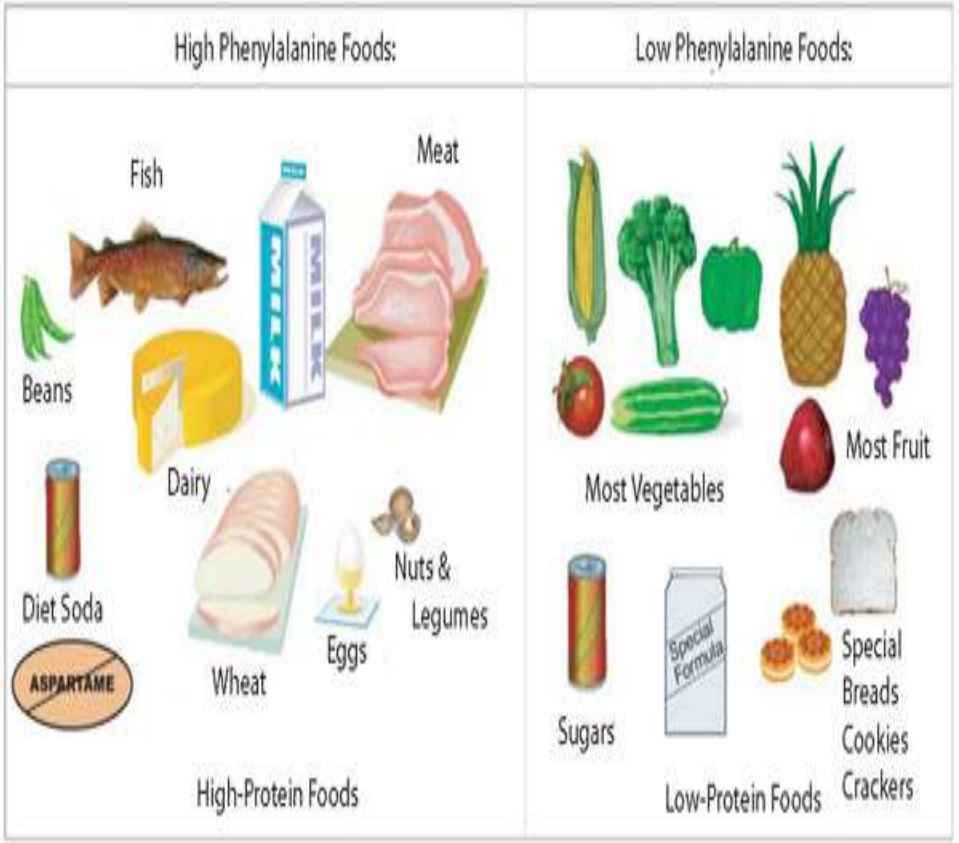
- **Phenylalanine** cannot be converted to **tyrosine**, and accumulates in plasma and is excreted in the urine with its metabolites, such as **phenyl pyruvic acid** (a phenyl ketone).



Phenylketonuria

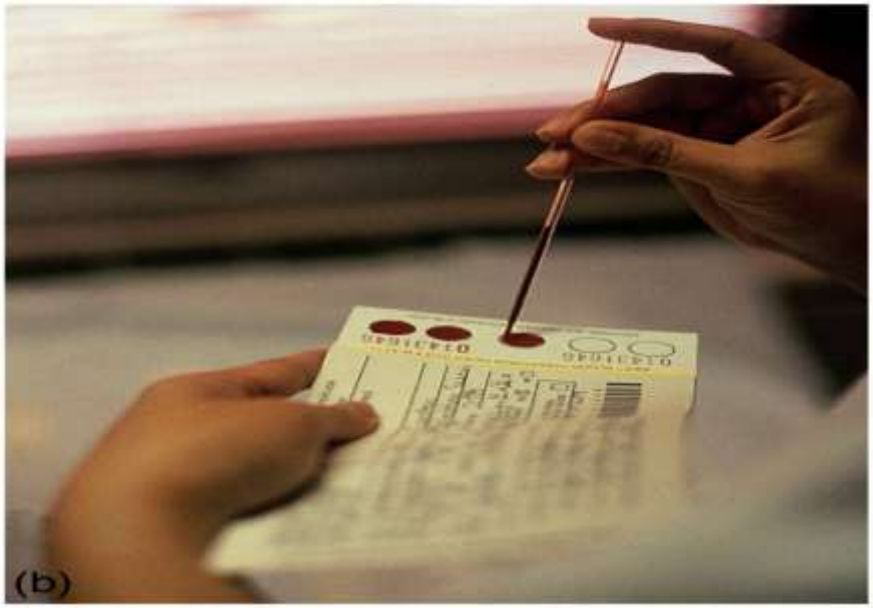
The clinical features include:

intellectual disabilities developing at between 4& 6 months, with psychomotor irritability, a tendency to reduced melanin formation because of reduced production of tyrosine – many patients are pale skinned, fair haired and blue eye, irritability, feeding problems, vomiting and fits during the first few weeks of life, often generalized eczema.



Phenylketonuria

- **Diagnosis** may involve measuring the **phenylalanine concentration** in blood taken from **a heel prick**.
- The **microbiological Guthrie test** was used to assay phenylalanine, but now many laboratories use chromatography methods or tandem mass spectroscopy.
- In the newborn, and especially in preterm infants, the enzyme system may not be fully developed and false positive results are likely if the test is performed too early. If a positive result is found, the test should be repeated later, to allow time for development of the enzyme.
- **The phenylalanine concentrations may be greater than 240 µmol/L.**



Microbiological Guthrie test

- A small drop of blood is taken from the heel of a newborn and applied to a card.
- In the original form of the test, a punch-out of the dried disc was incubated on a petri dish plated with bacteria **(*Bacillus subtilis*)** in the presence of a growth inhibitor**(B-2-thienyl-alanine)**.
- High levels of **Phe** in the blood sample overcome the inhibition, and allow the bacteria to grow.

Phenylketonuria treatment

- The aim of treatment is to **lower plasma phenylalanine** concentrations by giving a low-phenylalanine diet.
- Such treatment should be monitored carefully, especially if the patient is planning to conceive or is pregnant.
- Remember that the **artificial sweetener** aspartame is metabolized to phenylalanine.

- Tyrosinaemia

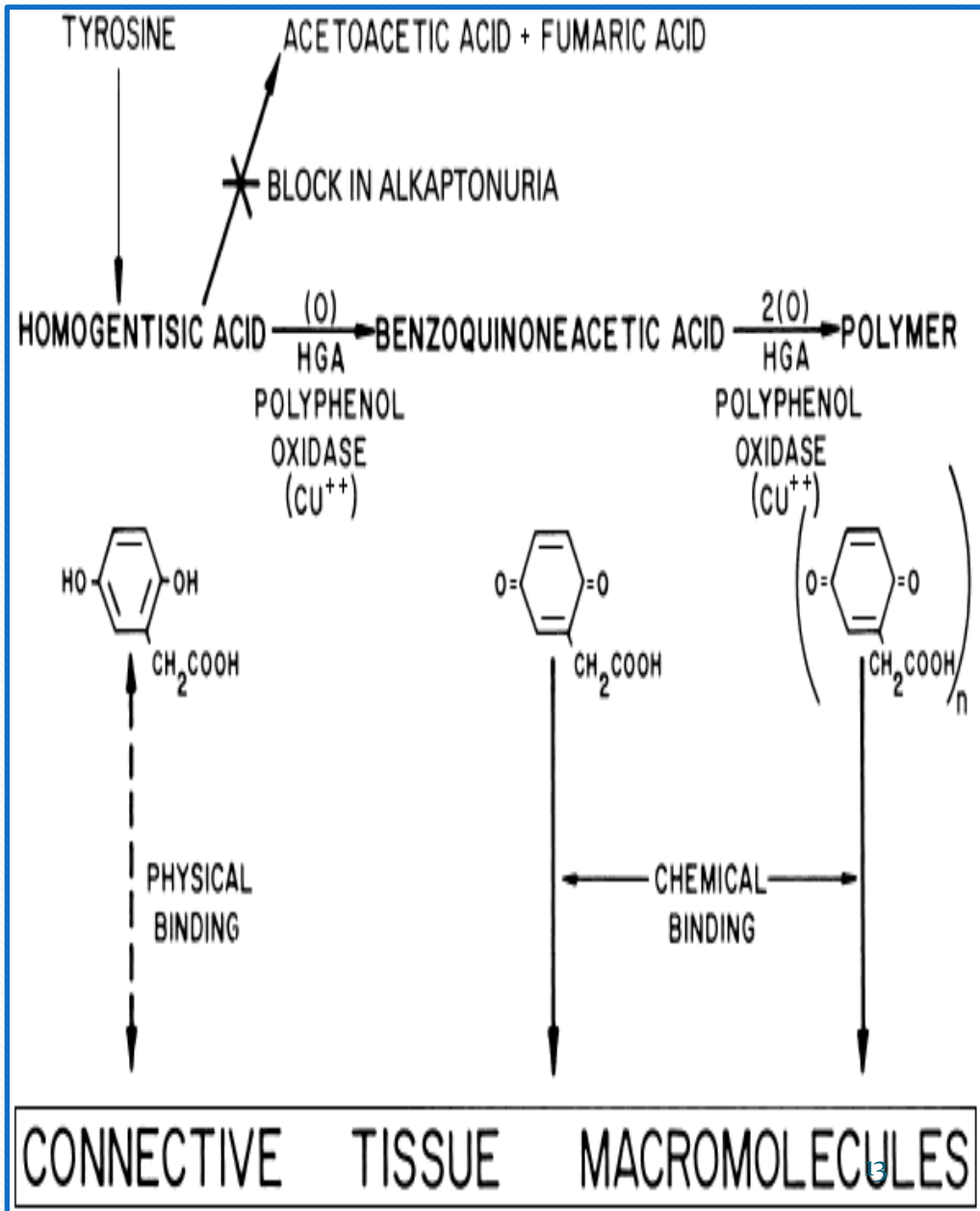
Enzyme	Defect	Major manifestations
Tyrosine aminotransferase	Tyrosinemia type II (oculocutaneous tyrosinemia)	Corneal thickening, developmental delay, hyperkeratosis of palms and soles
4-hydroxy phenylpyruvate dioxygenase	Transient tyrosinemia of the newborn	Transient immaturity of enzyme, usually resolves spontaneously
	Hawkinisinuria	Abnormal function of enzyme results in metabolic acidosis and failure to thrive in some patients
	Tyrosinemia type III	Primary deficiency of enzyme; asymptomatic to severe mental retardation and neurologic abnormalities
Homogentisate oxidase	Alcaptonuria	Arthritis in older patients Dark urine when exposed to air
Maleylacetoacetate isomerase		Reported in two siblings with liver failure and renal disease
Fumarylacetoacetate hydroxylase	Hepatorenal tyrosinemia Tyrosinemia type I	Liver, renal, and neurologic disease

Tyrosinaemia

- Treatment can be dietary, by liver transplantation or by nitro-trifluoro methyl benzoyl cyclohexane dione, which is thought to reduce the accumulation of some of the toxic metabolites.

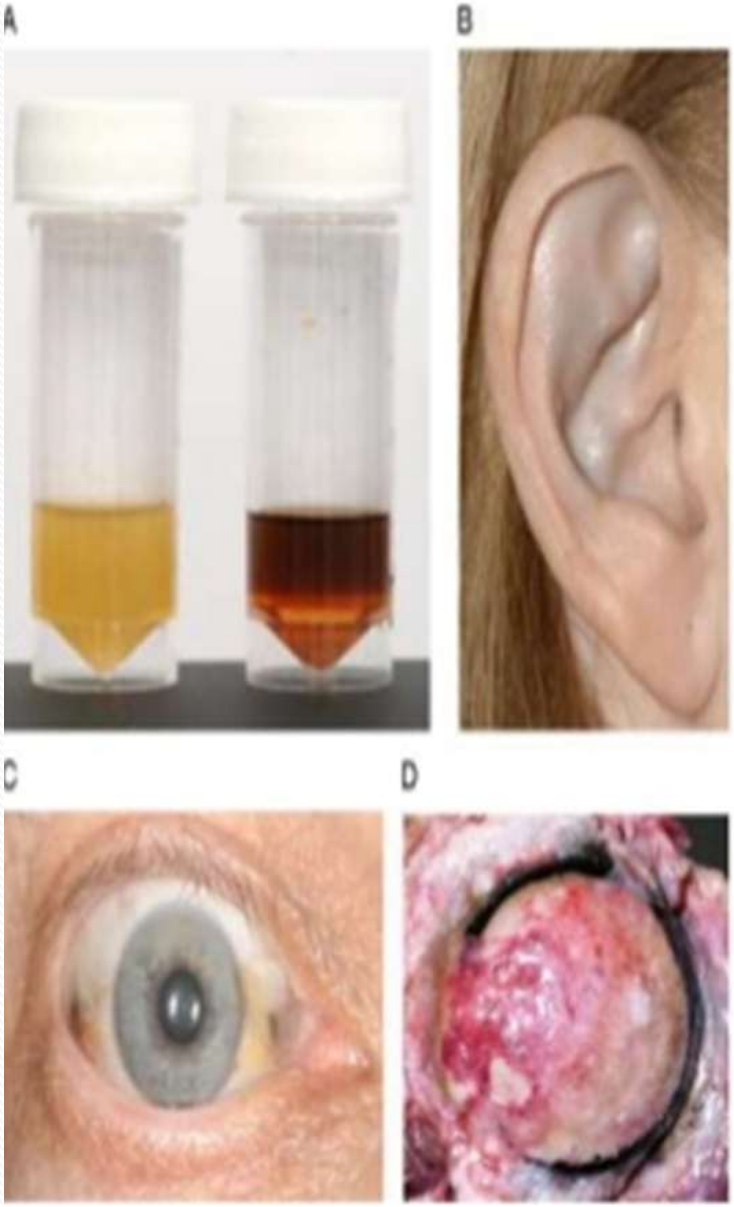
Alkaptonuria

- Alkaptonuria is an **autosomal recessive** disorder associated with a **deficiency of homogentisic acid oxidase**.
- **Homogentisic acid** accumulates in **tissues** and **blood**, and is passed in the **urine**.
- Oxidation and polymerization of homogentisic acid produce the **pigment alkapton**, in much the same way as polymerization of dihydroxy phenylalanine results in melanin.



Alkaptonuria

- The deposition of **alkapton** in cartilages, with consequent darkening, is called **ochronosis** and results in visible darkening of the cartilages of the ears and often arthritis in later life.
- The **conversion of homogentisic acid to alkapton** is accelerated in **alkaline conditions**, and sometimes the most obvious abnormality in alkaptonuria is darkening of the urine as it becomes more alkaline on standing.

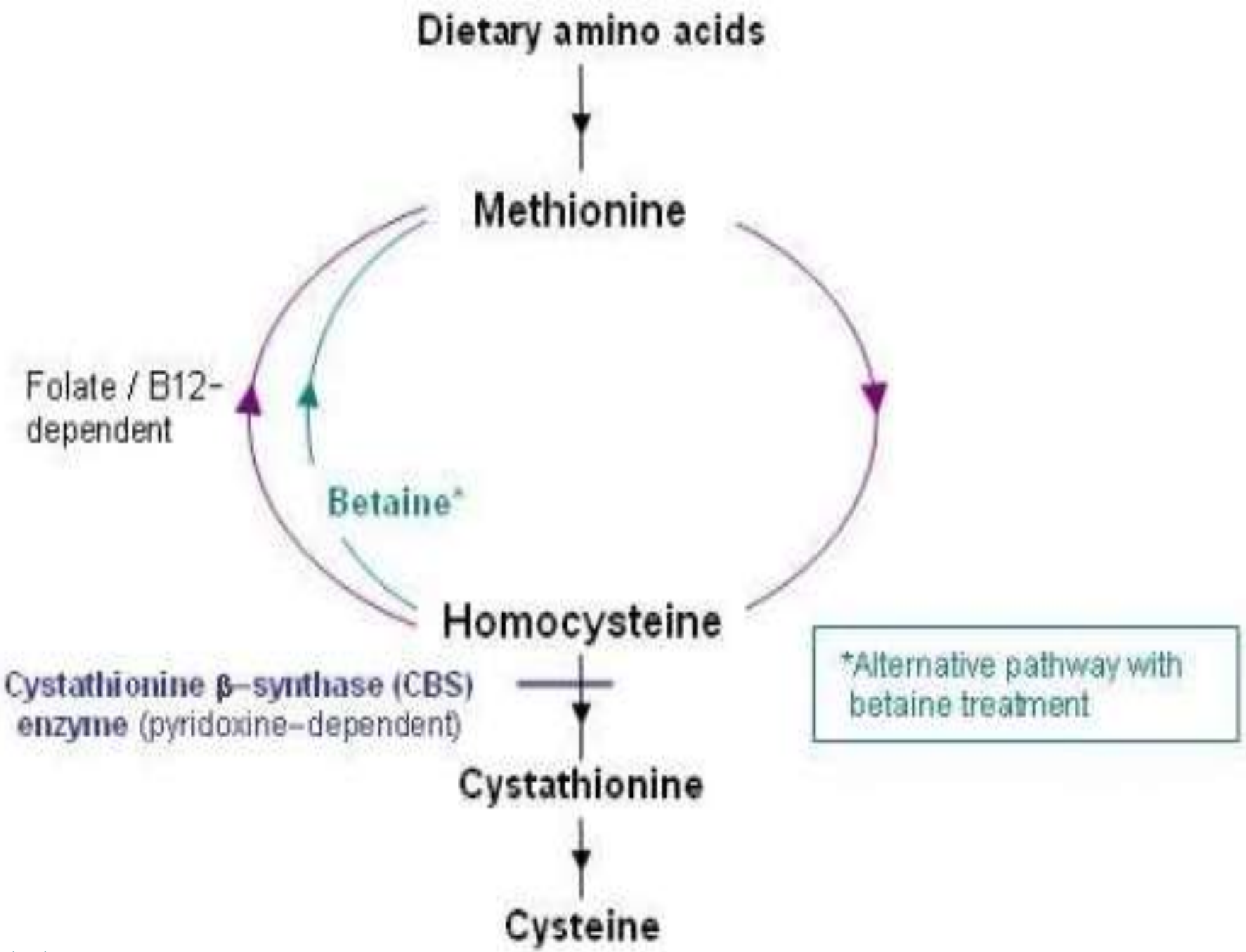


Albinism

- A deficiency of **tyrosinase in melanocytes** causes one form of albinism; it is inherited as an **autosomal recessive** disorder.
- Pigmentation of the skin, hair and iris is reduced and the eyes may appear pink.
- Reduced pigmentation of the iris causes **photosensitivity**, and decreased skin pigmentation is associated with an increased incidence of certain **skin cancers**.
- The **tyrosinase** involved in catecholamine synthesis is a different isoenzyme, controlled by a different gene; consequently, adrenaline (epinephrine) metabolism is normal.

Homocystinuria

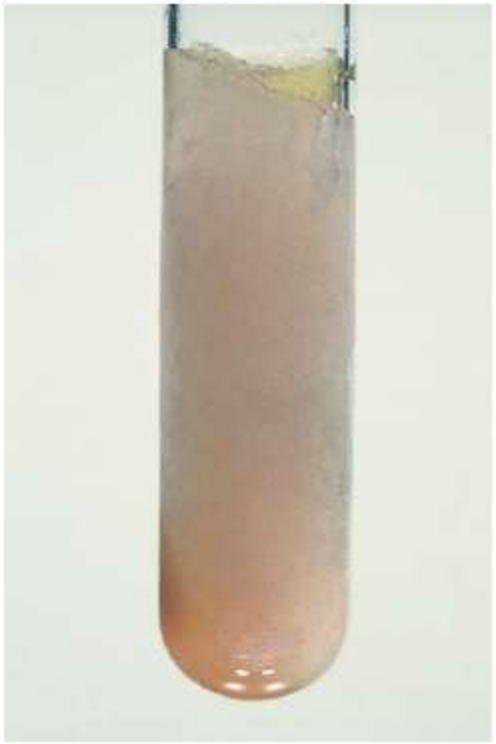
- Homocystinuria is an **autosomal recessive** disorder due to **deficiency of cystathionine synthase**.
- Patients may show progressive (CNS) dysfunction, thrombotic disease, eye disease, including cataracts, and cardiovascular problems.
- The **diagnosis of homocystinuria** is based on the presence of raised **urinary and plasma homocysteine** with low plasma **methionine concentrations**.
- The **defective enzyme** can be assayed in **cultured skin fibroblasts.**

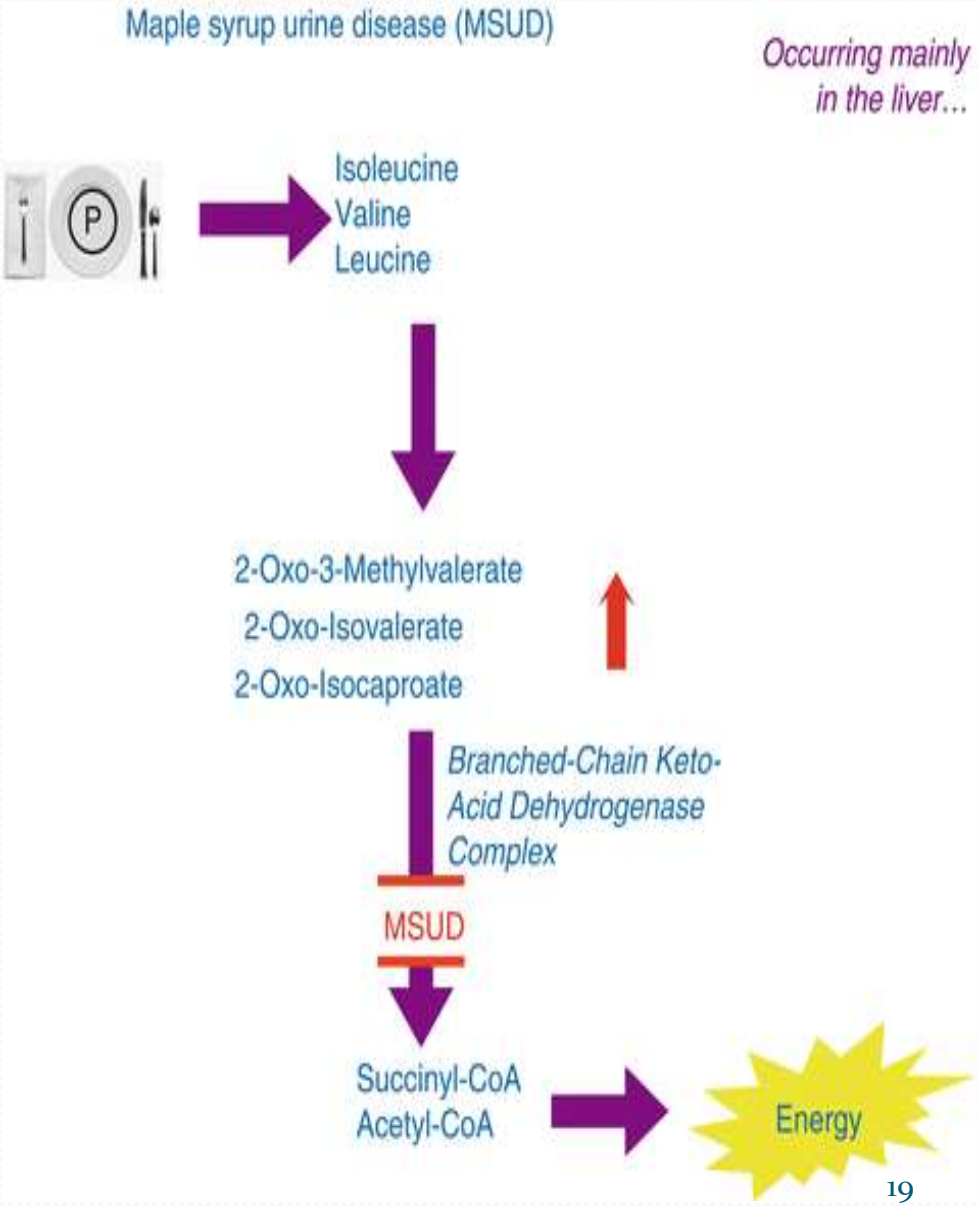

Dietary amino acids
Methionine
Folate / B12-dependent
Betaine*
Homocysteine
Cystathionine β–synthase (CBS) enzyme (pyridoxine–dependent)
Cystathionine
Cysteine
*Alternative pathway with betaine treatment

Maple syrup urine disease

- In **maple syrup urine disease**, which is inherited as an **autosomal recessive condition**, there is deficient **decarboxylation of the oxoacids** resulting from **deamination** of the **3 branched-chain a a.**
- These amino acids accumulate in the plasma and are excreted in the urine with their corresponding oxoacids. The **sweet smell** of the urine is like that of maple syrup.
- The disease presents **during the first week of life** and, if not treated, severe neurological lesions develop which cause death within a few weeks or months.
- The diagnosis of maple syrup urine disease is made by demonstrating raised concentrations of **branched-chain a a** in **plasma and urine** and **low plasma alanine concentration**. It may be confirmed by demonstrating the enzyme defect in leucocytes.

Maple syrup urine disease

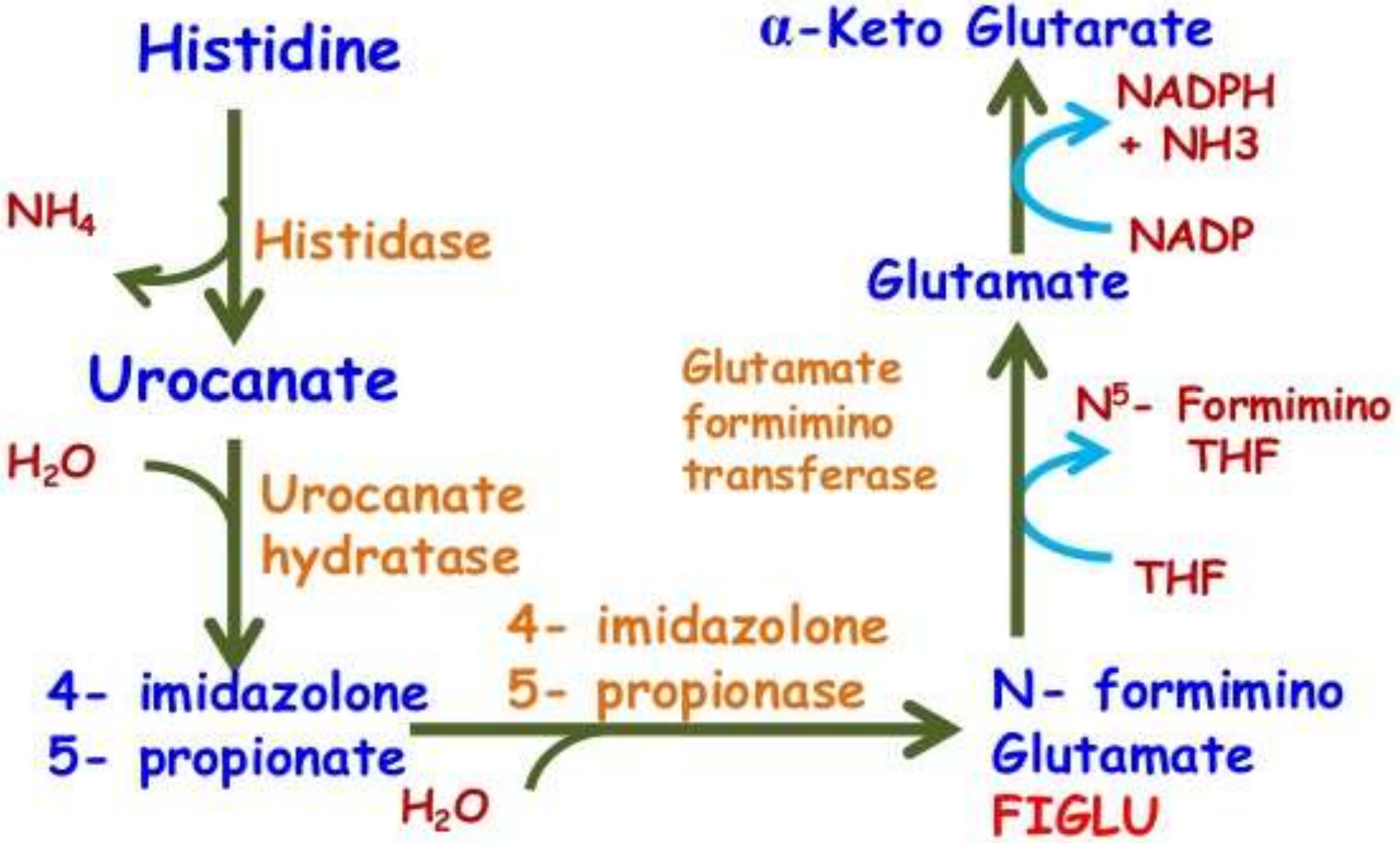




Histidinaemia

- Histidinaemia is associated with deficiency of **histidinase**, an enzyme needed for normal histidine metabolism, and is probably **inherited as an autosomal recessive trait**.
- Some individuals may have intellectual disabilities and speech defects, but others may be normal.
- **The diagnosis** is made by demonstrating **raised** plasma levels of **histidine**, and by finding histidine and the metabolite **imidazole pyruvic acid** in the urine, and the diagnosis of folic acid def. by histidine load test

Metabolism of Histidine



Inherited disorders of amino acid transport mechanisms

- Groups of chemically similar substances are often transported by shared or inter-related pathways.
- Such group-specific mechanisms usually affect **transport across all cell membranes,** and defects often involve **both the renal tubules and intestinal mucosa.**
- **Ex. Hartnup's disease, familial iminoglycinuria**

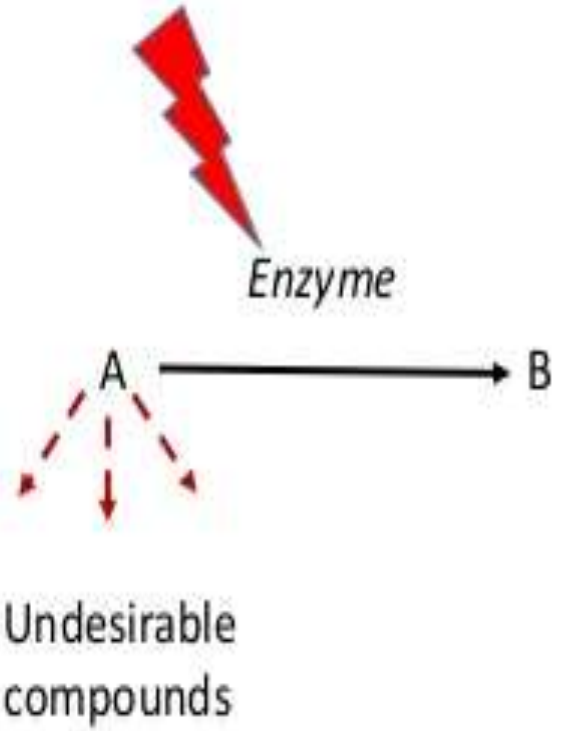
AMINOACIDURIA's

Overflow aminoaciduria

- Decreased synthesis of metabolite
- Accumulation of substrate
- Conversion of the substrate to undesirable compounds

Renal aminoaciduria

- Defects in single transport system
- Generalized tubular damage



Aminoaciduria

- **Specific aminoaciduria** is due to increased excretion of either a single amino acid or a group of chemically related amino acids. It may be overflow or renal in type

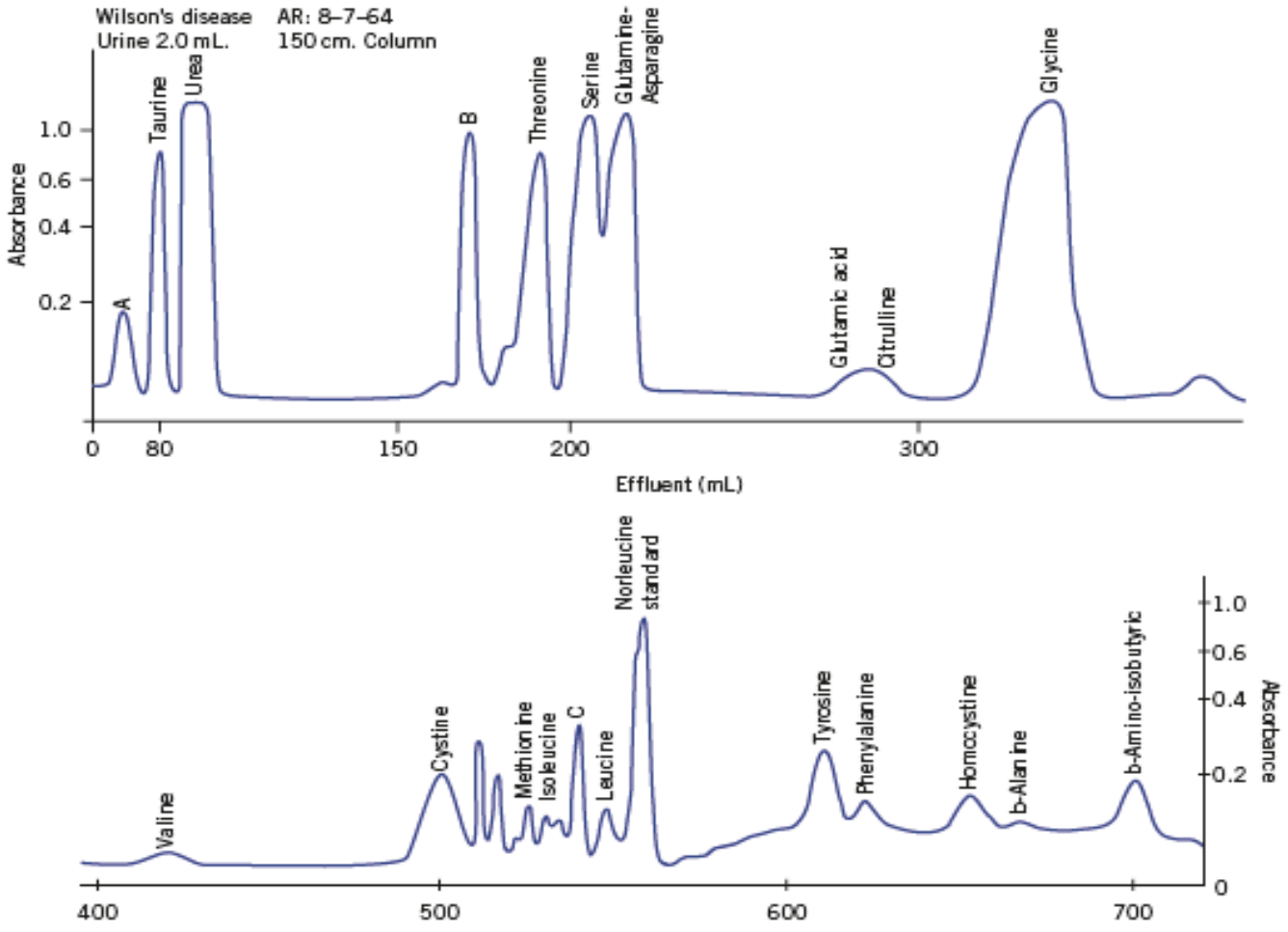
- **Non-specific aminoaciduria**, in which there is increased excretion of a number of unrelated amino acids, is almost always due to an acquired disorder.

Aminoaciduria

- It may be overflow in type, as in **severe hepatic disease** when **impaired deamination of amino acids** causes raised plasma concentrations; more commonly, renal aminoaciduria results from non-specific proximal tubular damage, and other substances that are usually almost completely reabsorbed by the proximal tubule are also lost (**phosphogluco aminoaciduria; Fanconi's syndrome**).
- If it occurs due to an inborn error of metabolism, it is rarely a direct result of the genetic defect, but more commonly **secondary to tubular damage** caused by deposition of the substance not metabolized normally, such as copper in Wilson's disease.

Amino aciduria

Chromatographic pattern of amino aciduria.



Treatment of aminoaciduria depends on underlying cause. The treatment causes the reducing of the amino acids providing the body with a way to utilize the amino acids more efficiently, allowing the kidney to clear all the amino acids more effectively.

Treatment of aminoaciduria may include,

- Diet restrictions.

Reducing the amount of a particular amino acids in the diet. Example Phenylalanine.

Inborn errors of the dibasic amino acids

- **cystine, ornithine, arginine and lysine** – (COAL)
- **Cystinuria** is the result of an **autosomal recessive** inherited abnormality of **tubular reabsorption**, with **excessive urinary excretion**, of the dibasic amino acids.
- A **similar transport defect** has been demonstrated in the **intestinal mucosa**, but, although dibasic amino acid absorption is reduced, **deficiencies do not occur** because they can be synthesized in the body.

Cystinuria

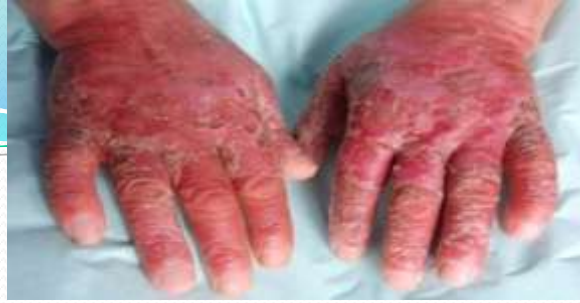
- Cystine is relatively insoluble and, because of the **high urinary concentrations in homozygotes**, may precipitate and form calculi in the renal tract. In **heterozygotes,** increased excretion can be demonstrated, but concentrations are rarely high enough to cause precipitation.
- **The diagnosis** of cystinuria is made by demonstrating excessive urinary excretion of the characteristic amino acids. All these amino acids must be identified to distinguish this from cystinuria occurring as part of a generalized aminoaciduria.
- **The management** of cystinuria aims to prevent calculi formation by reducing urinary concentration. The patient should drink plenty of fluid. Alkalinizing the urine increases the solubility of cystine. If these measures prove inadequate, D-penicillamine may be given; this forms a chelate, which is more soluble than cystine alone

Cystinosis

- This is a very rare but serious disorder of cystine metabolism, characterized by intracellular accumulation and **storage of cystine in many tissues**.
- It must be distinguished from cystinuria, a relatively harmless condition. **Renal tubular damage** by cystine causes **Fanconi's syndrome.**
- **Amino aciduria** is non-specific and of renal origin. Affected individuals may die young

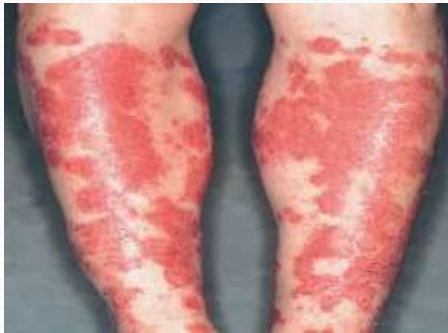
Hartnup's disease

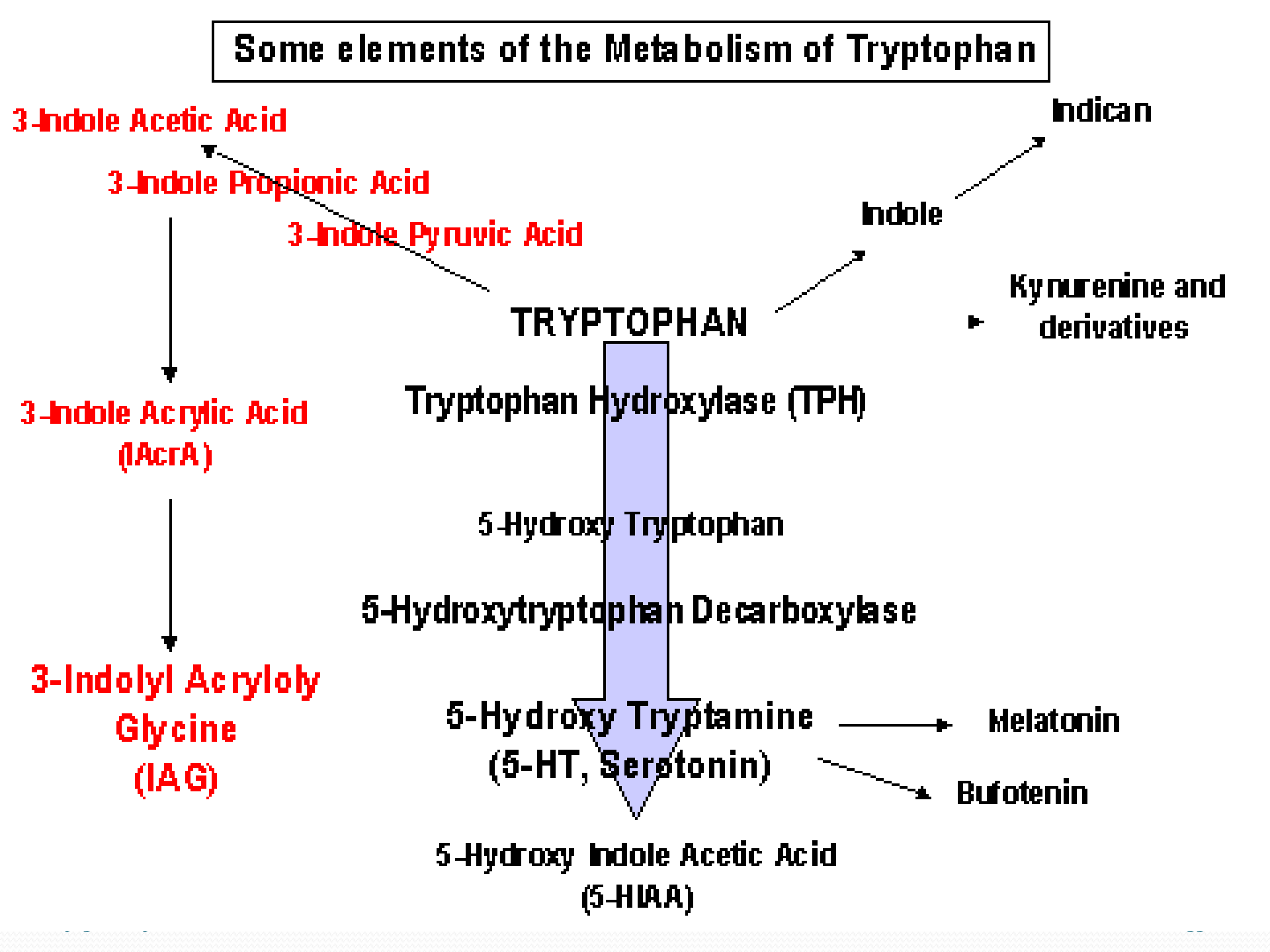
- Hartnup's disease is a **rare autosomal recessive disorder.**
- Many of the clinical manifestations can be ascribed to reduced intestinal absorption and **increased urinary loss of tryptophan**.
- This amino acid is normally partly converted to **nicotinamide**, the conversion being especially important if the dietary intake of nicotinamide is low.



Hartnup's disease

- The clinical features are intermittent and resemble those of pellagra, namely a red, scaly rash on exposed areas of skin, reversible cerebellar ataxia and mental confusion of variable degree.
- **Excessive amounts of indol compounds**, originating from bacterial action on unabsorbed tryptophan, are absorbed from the gut and excreted in the urine.




Some elements of the Metabolism of Tryptophan
3-Indole Acetic Acid
3-Indole Propionic Acid
3-Indole Pyruvic Acid
Indican
Indole
Kynurenine and derivatives
TRYPTOPHAN
Tryptophan Hydroxylase (TPH)
3-Indole Acrylic Acid (IAcrA)
5-Hydroxy Tryptophan
5-Hydroxytryptophan Decarboxylase
3-Indolyl Acryloly Glycine (IAG)
5-Hydroxy Tryptamine (5-HT, Serotonin)
Melatonin
Bufotenin
5-Hydroxy Indole Acetic Acid (5-HIAA)

Familial iminoglycinuria

- Increased urinary excretion of the imino acids proline and hydroxyproline and glycine; despite normal plasma concentrations, is due to a transport defect for these 3 aa.
- The condition is inherited as **an autosomal recessive trait.** It is apparently harmless, but must be differentiated from other more serious causes of iminoglycinuria, such as the defect of proline metabolism, hyperprolinaemia.

Inborn errors

Clinical chemistry part III ٢٠١٩

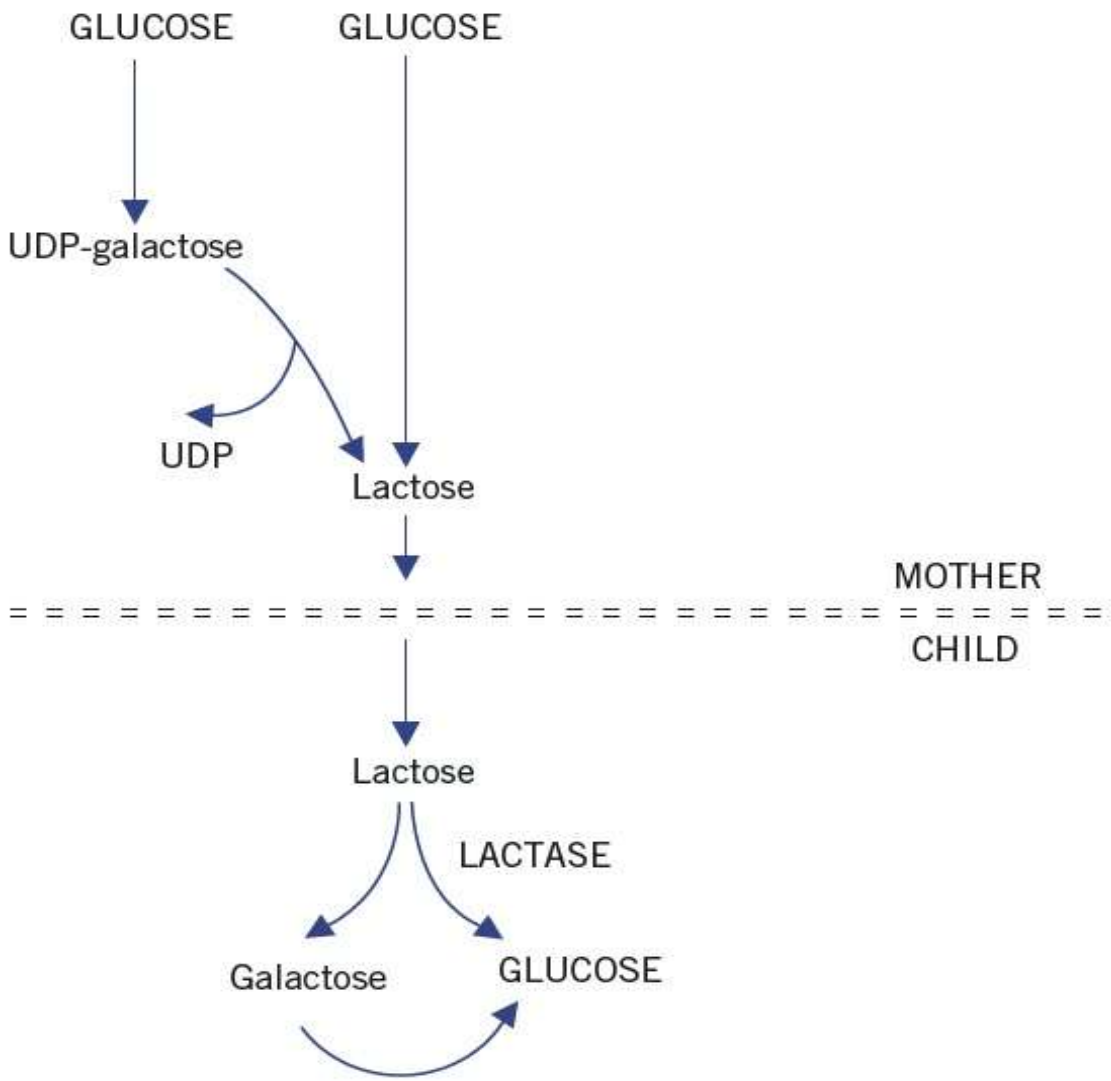
Disorders of sugars

Galactosaemia is an autosomal recessive disorder due to galactose-1-phosphate uridyl transferase **(Gal-1-P UT) deficiency.**

Galactose is necessary for the formation of **cerebrosides, some glycoproteins and milk.**

- Excess is rapidly converted into glucose.
- The symptoms of galactosaemia become apparent only if the infant is taking milk; the plasma galactose concentrations then rise.

The main features of galactosemia are :



- Vomiting and diarrhea.
- Prolonged prothrombin time.
- Hepatosplenomegaly with jaundice and cirrhosis.
- Cataract formation.
- Intellectual disabilities.
- Renal tubular damage due to the deposition of **gal-1-p** in the tubular cells (Fanconi's syndrome).

Diagnosis:

- **Galactose** is a reducing substance. The urine may give a positive reaction with **Clinitest tablets**; this feature may be absent if the subject is not receiving milk and therefore galactose.
- **Tubular damage** may cause a generalized **amino aciduria.**
- The diagnosis is made by identifying galactose by **thin-layer chromatography** and by demonstrating a deficiency of **Gal-1-P UT activity in erythrocytes.**

Treatment

- Treatment involves eliminating galactose in milk and milk products from the diet. Sufficient galactose for the body's requirements can be synthesized endogenously as **uridyl disphosphate galactose.**
- This will **reverse the acute symptoms** but not some of the chronic long-term neurological complications.

The glycogen storage disorders

- *GSD **type I***

This is known as **von Gierke's disease** and is a deficiency of **glucose-6-phosphatase** Patients may display a lactic acidosis, hypoglycaemia, hyperuricaemia and hypertriglyceridaemia.

- *GSD **type II***

Pompe's disease or **maltase deficiency (α -1,4- glucosidase)** is a lyosomal defect. It is associated with skeletal myopathy, including muscular hypotonia and cardiomyopathy.

- *GSD **type III***

This is a defect of **debranching enzyme** and is known as **Forbes–Cori disease**. Abnormal glycogen with short external branches accumulates in skeletal muscle, heart and liver. This can result in growth retardation, muscular weakness and cardiomyopathy.

The glycogen storage disorders

- ***GSD type IV***

This is a defect of **glycogen branching enzyme** and is also called **Andersen's disease**. There is hepatosplenomegaly and also cardiac and skeletal muscle defects.

- ***GSD type V***

McArdle's disease is a deficiency of **muscle phosphorylase**. Muscle cramps and fatigue occur on heavy exertion. The urine may be burgundy-red in color due to **myoglobin** from muscle breakdown.

- ***GSD type VI***

Hers' disease is due to **hepatic phosphorylase** deficiency. Symptoms may be mild, although growth retardation may occur.

- ***GSD type VII***

Tarui's disease is due to **phosphofructokinase deficiency**. The symptoms are similar to those of type V.

Lipid disorders and organic acidurias

- **The organic acids were** derived from the metabolism of AA, CHO and lipids are often **detectable in the urine**; others **accumulate** if there is an enzyme deficiency in a specific metabolic pathway.

Examples include

- **Methyl malonic acidaemia,**
- **Glutaric acidaemia,**
- **Isovaleric acidaemia and**
- **proprionic acidaemia.**

These disorders, known as organic acidurias, are individually rare, but collectively have an incidence of about **1 in 12 000 births.**

Signs& Symptoms

- They may present
  in the **neonatal period**
  life-threatening metabolic acidosis,
- vomiting and
- hypotonia,
  in **early infancy**
  failure to thrive,
- a Reye-like syndrome and
- convulsions associated with profound hypoglycemia.
- They may also be a cause of sudden infant death.

Lipid disorders and organic acidurias

- **Medium-chain acyl coenzyme A dehydrogenase** deficiency is autosomal recessive and is one of the most common **fatty acid oxidation** defects (about 1 in 10 000 live births).

- This potentially fatal condition may present with **hypoketotic hypoglycaemia**, **encephalopathy, seizures** and **hepatomegaly** following **diarrhea and vomiting** (reduced food intake).
- **Urinary dicarboxylic aciduria** with **glycine** conjugates may occur, along with increased plasma **octanoyl carnitine** (acylcarnitine).

Mitochondrial disorders

- **Mitochondrial DNA** (mt DNA) is derived from the mother. This differs from nuclear DNA in that there are no **introns and replication** of mt DNA, and the mutation rate is about **10–100 times** greater than that of nuclear DNA.

- **Mitochondria** lack an adequate DNA repair mechanism.
- A number of **clinical features** may be present, including neuropathy, intellectual disabilities, lactic acidosis, myopathy, ocular defects, DM, anaemia and hearing loss.

- There are a number of mitochondrial disorders, including **Leigh's syndrome**, MELAS syndrome (**m**itochondrial **e**ncephalomyopathy, **l**actic **a**cidosis, **s**troke), **Kearns–Sayre syndrome**, NARP syndrome (**n**europathy, **a**taxia, **r**etinitis **p**igmentosa) and LHON syndrome (**Leber's hereditary optic neuropathy**).

Mitochondrial disorders

- The arterial or venous **lactate to pyruvate ratio** may be high (more than 50:1), which suggests a **metabolic block in the respiratory chain system.**
- There is often a **high plasma lactate at rest. Plasma creatine kinase** activity may be raised and **rhabdomyolysis** can occur with **myoglobinuria.**
- Specialized **muscle histology** may be useful and also **genetic tests** and **family studies**.

Peroxisomal disorders

1- Deficiency of a peroxisomal enzyme

2- Defect in forming intact peroxisomes.

There are probably about 20 of these disorders affecting about **1 in 30 000** individuals. Peroxisomes are involved in a number of **metabolic processes.**

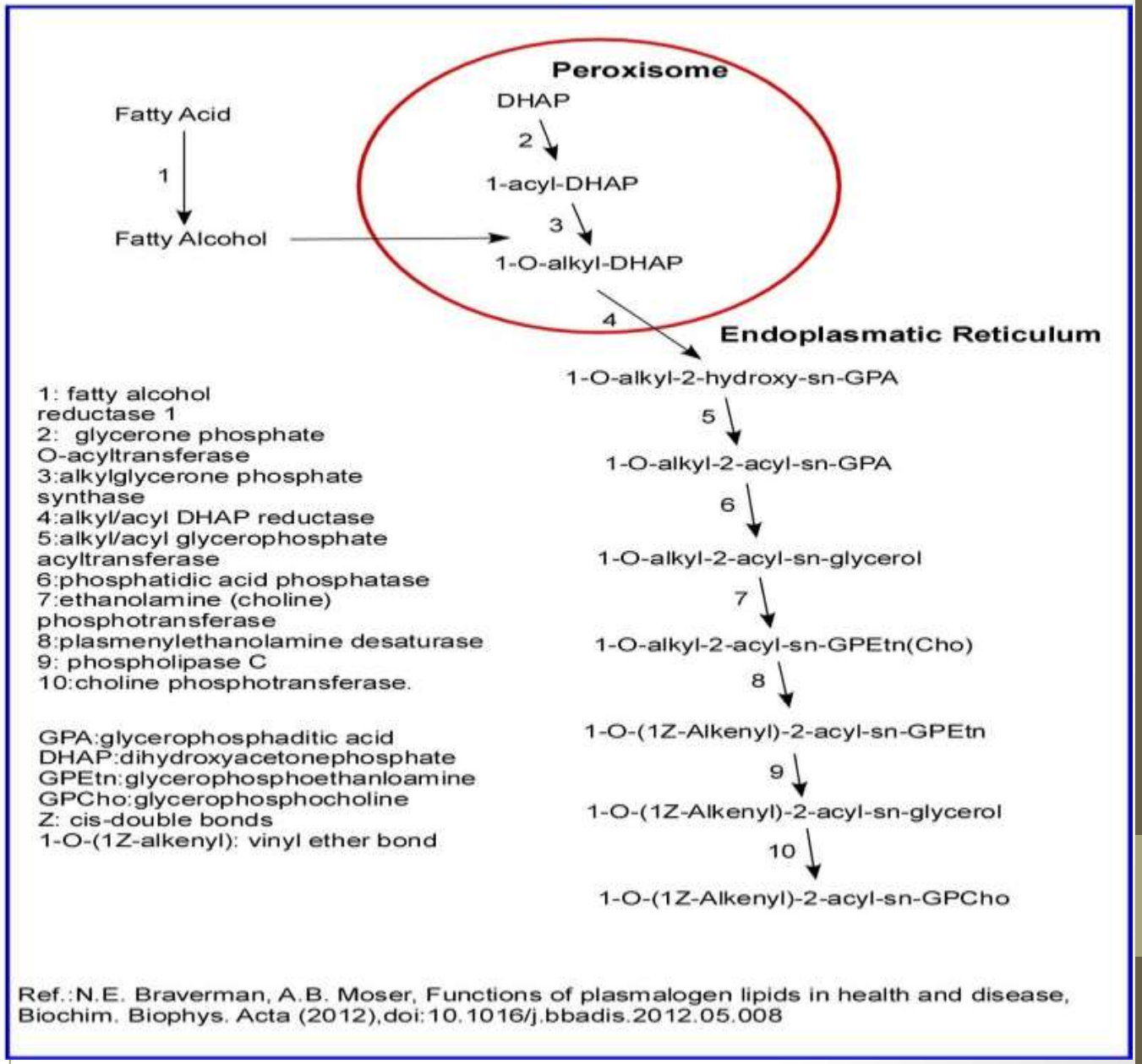
Examples: defects of phytanic acid oxidation (Refsum's disease), dihydroxyacetone phosphate acyltransferase abnormality (Zellweger's syndrome), catalase defects (neonatal adrenoleucodystrophy), and abnormal plasmalogen biosynthesis (rhizomelic chondrodysplasia punctata).

- These conditions may present with a variety of features, including dysmorphia, cataracts, liver disease, retinitis pigmentosa, adrenal insufficiency, peripheral neuropathy, deafness and ataxia.

Rhizomelic chondrodysplasia punctata

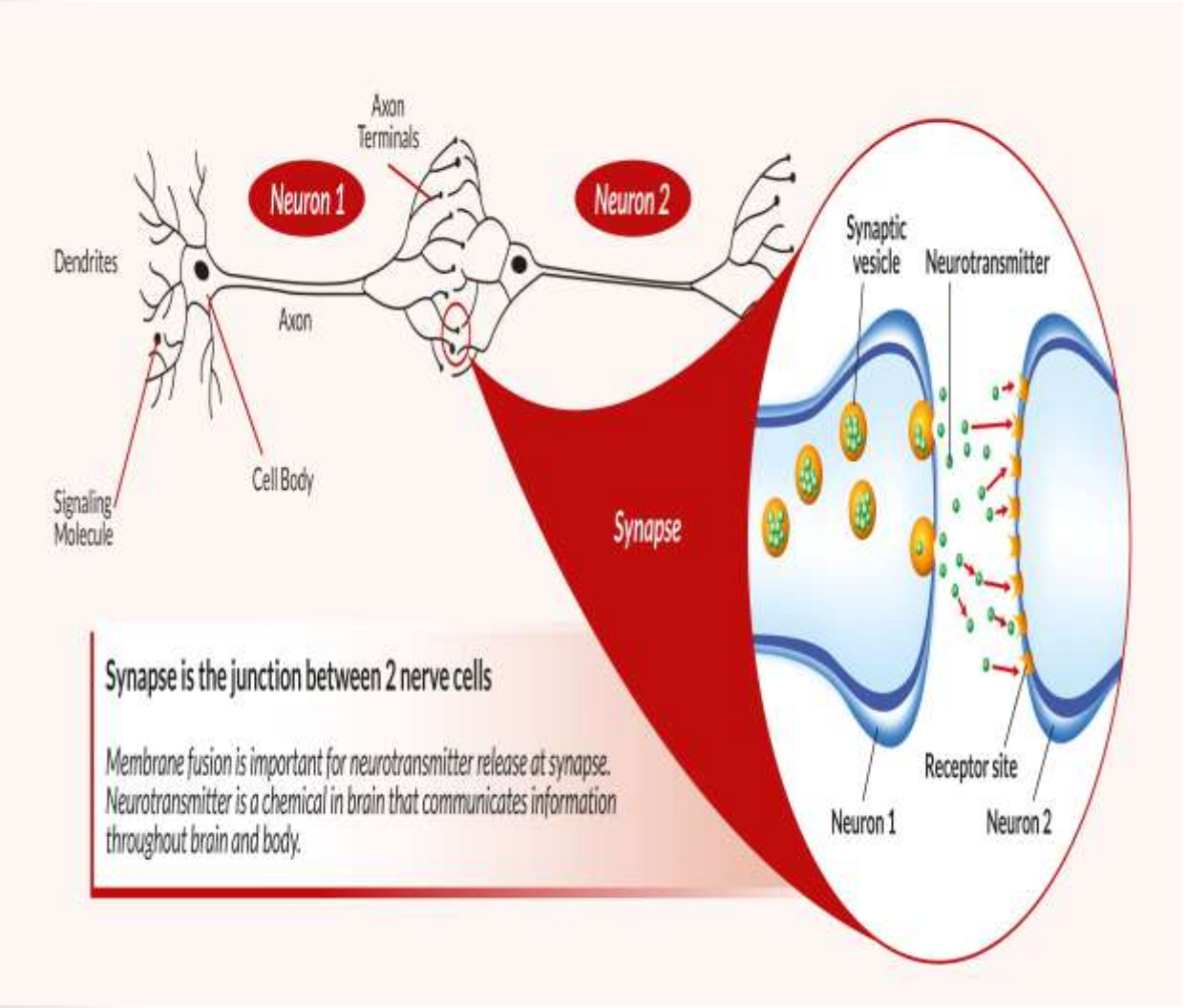
- is a rare developmental brain disorder characterized by systemic shortening of the proximal bones (rhizomelia), seizures, recurrent respiratory tract infections and congenital cataracts.
- The affected individuals have **low levels of plasmalogens**

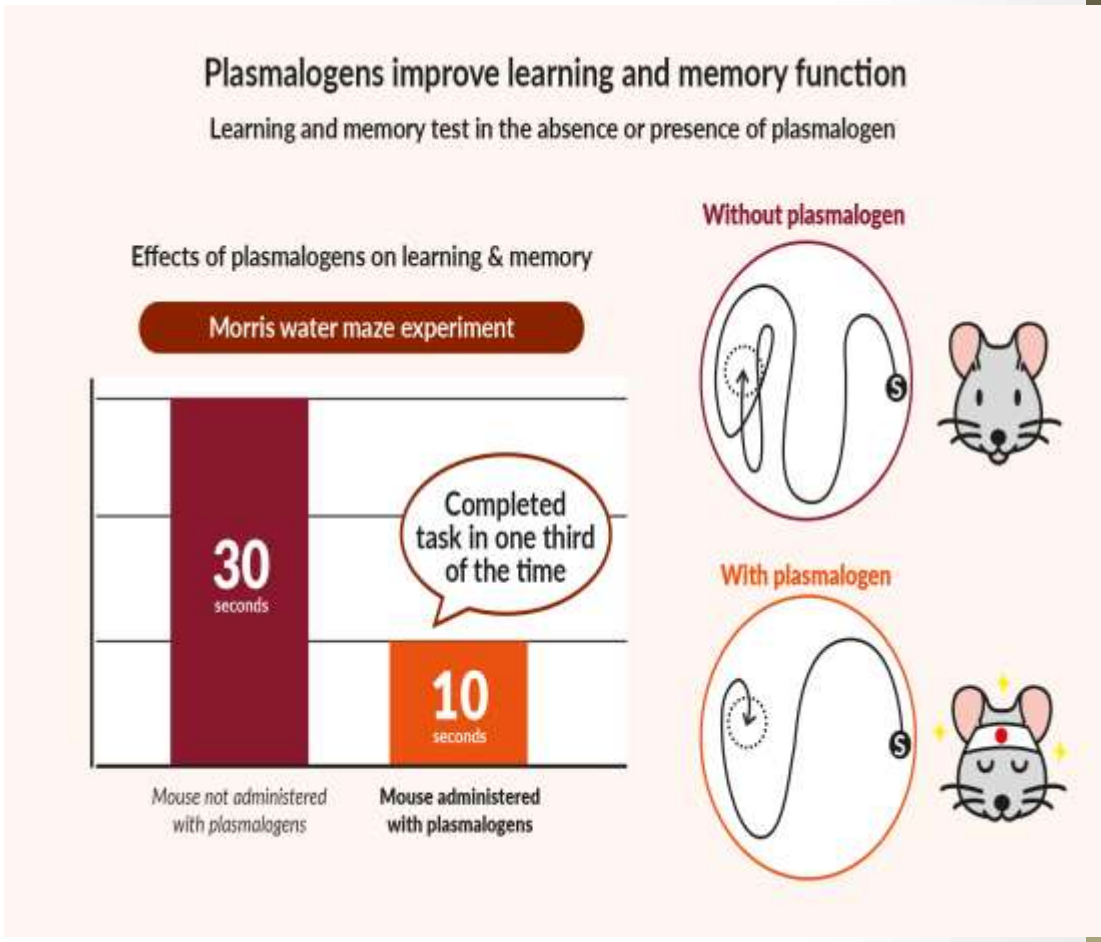
plasmalogen biosynthesis



Ref.:N.E. Braverman, A.B. Moser, Functions of plasmalogen lipids in health and disease, Biochim. Biophys. Acta (2012),doi:10.1016/j.bbadis.2012.05.008

Plasmalogen function





Drugs and inherited metabolic disorders

- The variation in individual response to drugs may be partly due to genetic variation. There are a number of well-defined inherited disorders that are aggravated by, or which become apparent only after, the administration of certain drugs.

These disorders may be classified into **two groups**.

- Disorders resulting in **deficient metabolism of a drug.**
- Disorders resulting in **an abnormal response to a drug**

Disorders resulting in deficient metabolism of a drug

- The **muscle relaxant suxamethonium** (succinyl choline, or scoline) normally has a very brief action because it is rapidly broken down by plasma **cholinesterase.**
- In **suxamethonium sensitivity:** a cholinesterase variant of low biological activity impairs the breakdown of the drug, and **prolonged postoperative respiratory paralysis** may result (‘**scoline apnoea’).**

- Two other inherited disorders are characterized by defective metabolism of the drugs **isoniazid and phenytoin**. In both, toxic effects occur more frequently, and at lower dosages, than in normal individuals.

Disorders resulting in an abnormal response to a drug

- Deficiency of **glucose-6-phosphate dehydrogenase (G6PD)** may cause hemolytic anaemia, and is relatively common in ethnic groups of Mediterranean origin. It is X-linked.
- This enzyme **catalyses** the first step in the **hexose monophosphate pathway** and is needed for the formation of **nicotinamide adenine dinucleotide phosphate**, which is important for the **maintenance of intact red cell membranes**.
- **Hemolysis** may be precipitated by certain **antimalarial drugs**, such as primaquine, and by **sulphonamides**.

- In the **inherited hepatic porphyrias**, acute attacks may be precipitated by various drugs, such as **barbiturates. In acute intermittent porphyrias when porphobilinogen deaminase**

Disorders resulting in an abnormal response to a drug

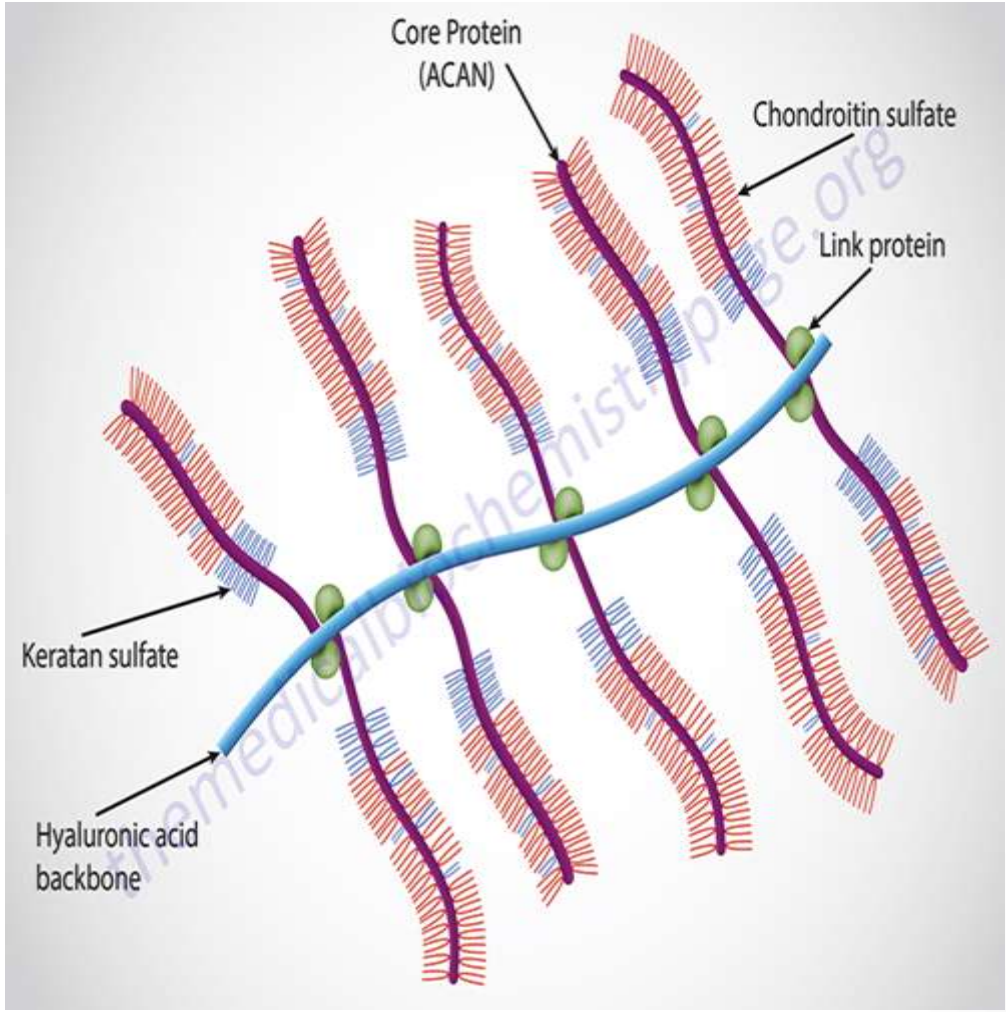
- Some people react to **general anaesthetics** (most commonly halothane with suxamethonium) with
- a rapidly rising temperature,
- muscular rigidity and
- acidosis (malignant hyperpyrexia),
- which is associated with high mortality.

- Many, **but not all,** susceptible subjects in affected families have a high plasma **creatine kinase activity.**

Lysosomal disorders

- The **mucopolysaccharidoses :**

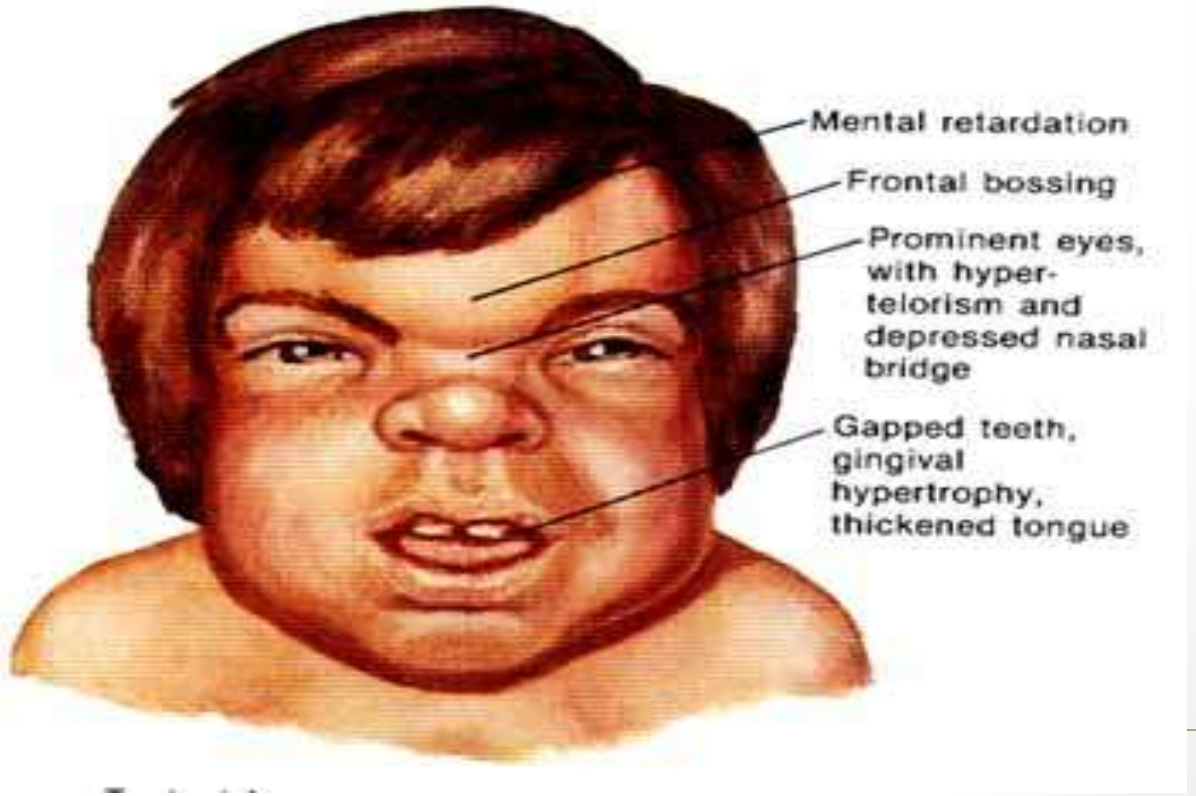
- The MPSs are rare conditions caused by defects of any of the several enzymes that hydrolyse mucopolysaccharides (glycosaminoglycans), which therefore accumulate in tissues such as the **liver, spleen, eyes, CNS, cartilage and bone.**



Hurler’s syndrome (MPS IH)

- - is the least rare, and is inherited as an **autosomal recessive disorder.**
- - Patients present in **infancy** or **early childhood due to a deficiency of alpha-L iduronidase** **an enzyme responsible for the degradation of GAGs in lysosomes** with the characteristic

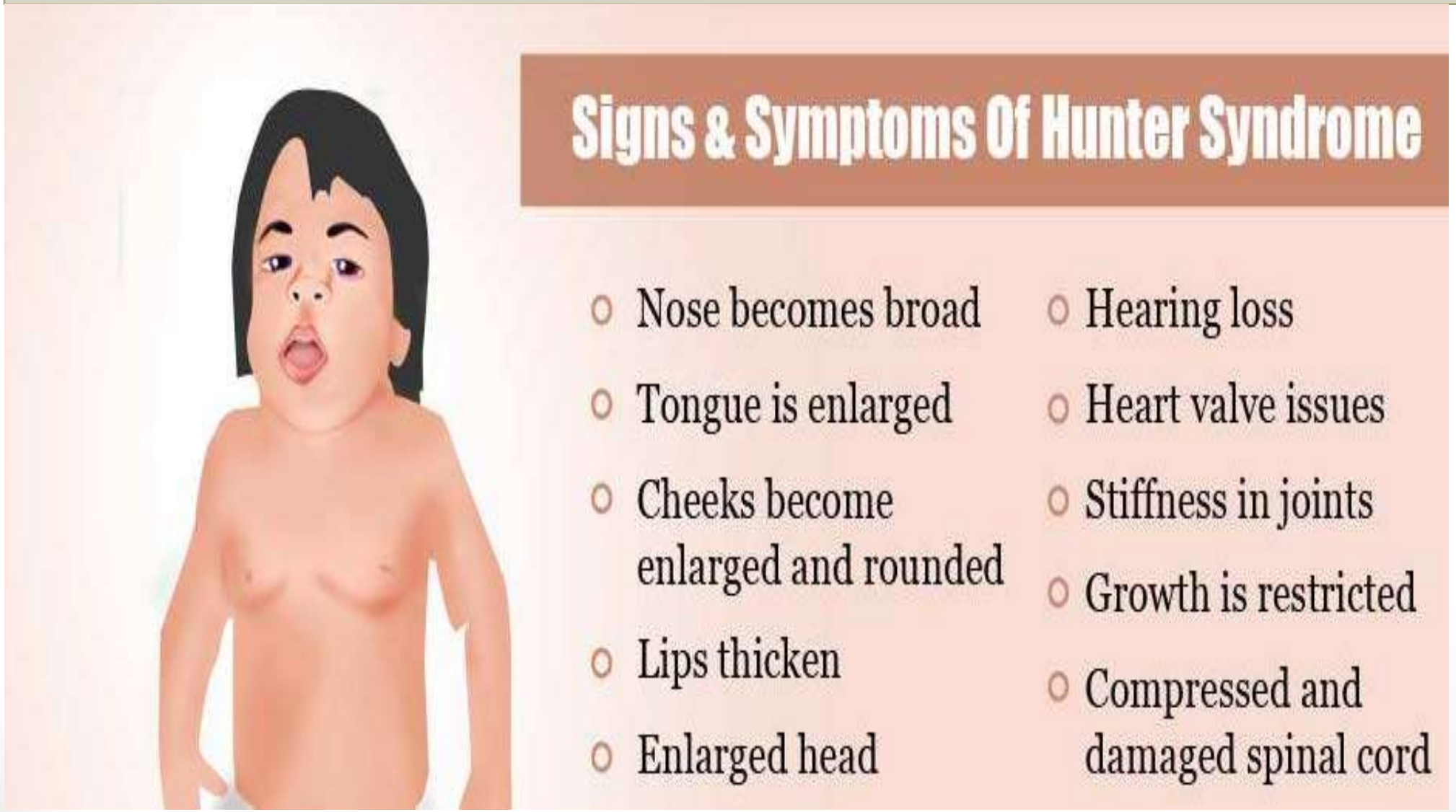
- coarse features,
- short stature,
- intellectual disabilities and
- clouding of the cornea.



Hunter’s syndrome (MPS II)

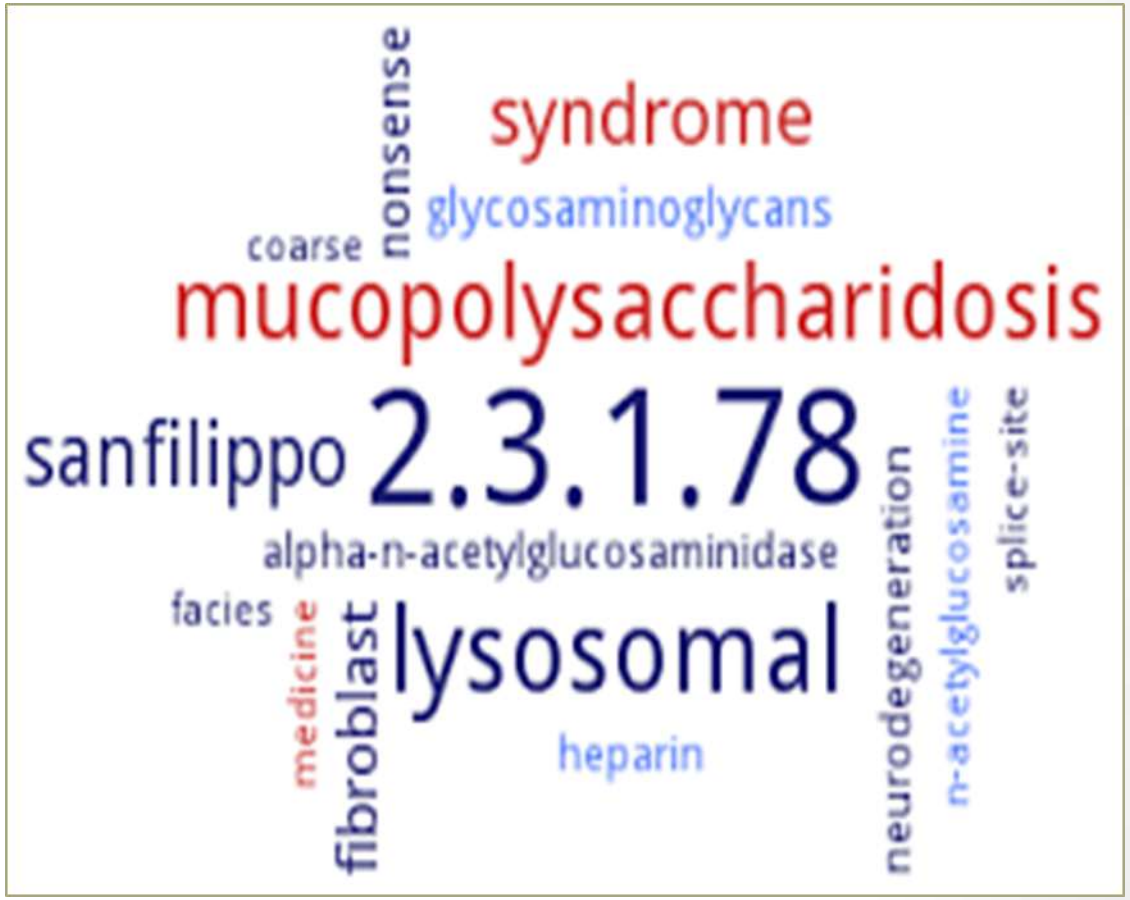
- Is a lysosomal storage disease caused by a deficiency of the lysosomal enzyme iduronate-2-sulfatase (I2S). The lack of this enzyme causes **heparan sulfate** and **dermatan sulfate** to accumulate in all body tissues.
- **Hunter syndrome** is the only MPS syndrome to exhibit X-linked recessive inheritance.
- Hunter syndrome causes abnormalities in many organs, including the skeleton, heart, and respiratory system.
- In severe cases, this leads to death during the teenage years. Unlike MPS I, corneal clouding is not associated with this disease.

Hunter syndrome



other mucopolysaccharide conditions

- **Sanfilippo's syndrome, (MPS-III)** is a rare autosomal recessive lysosomal storage disease.
- It is caused by a deficiency in one of the enzymes needed to break down the (GAG) **heparan sulfate** (which is found in the **extra-cellular matrix** and on **cell surface glycoproteins**)which manifests severe CNS abnormalities.



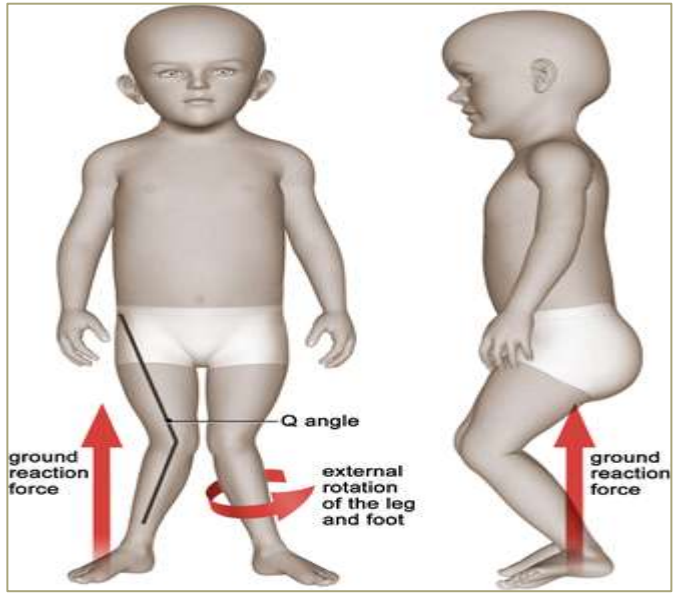
other mucopolysaccharide conditions

- **Morquio's syndrome (MPS IV)… accumulation of keratan sulfate.**

- **short stature**,
- barrel chest,
- **genu valgum** and
- other **skeletal abnormalities**.

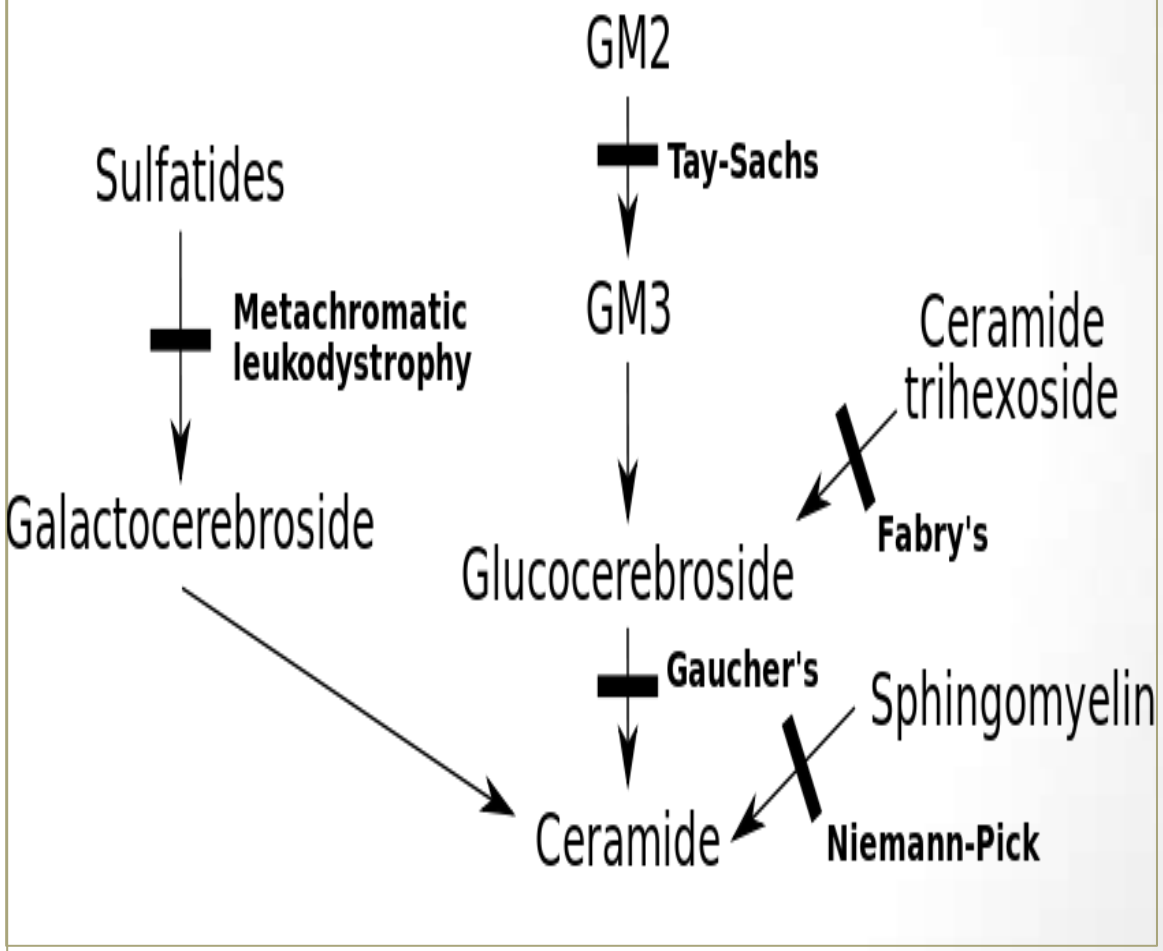
in which the body cannot process certain types of mucopolysaccharides (long chains of sugar molecules), which the body uses **as lubricants and shock absorbers.**

- The MPSs can initially be diagnosed by demonstrating increased urinary excretion of sulphated GAGs (dermatan, heparan and keratin) sulphates .



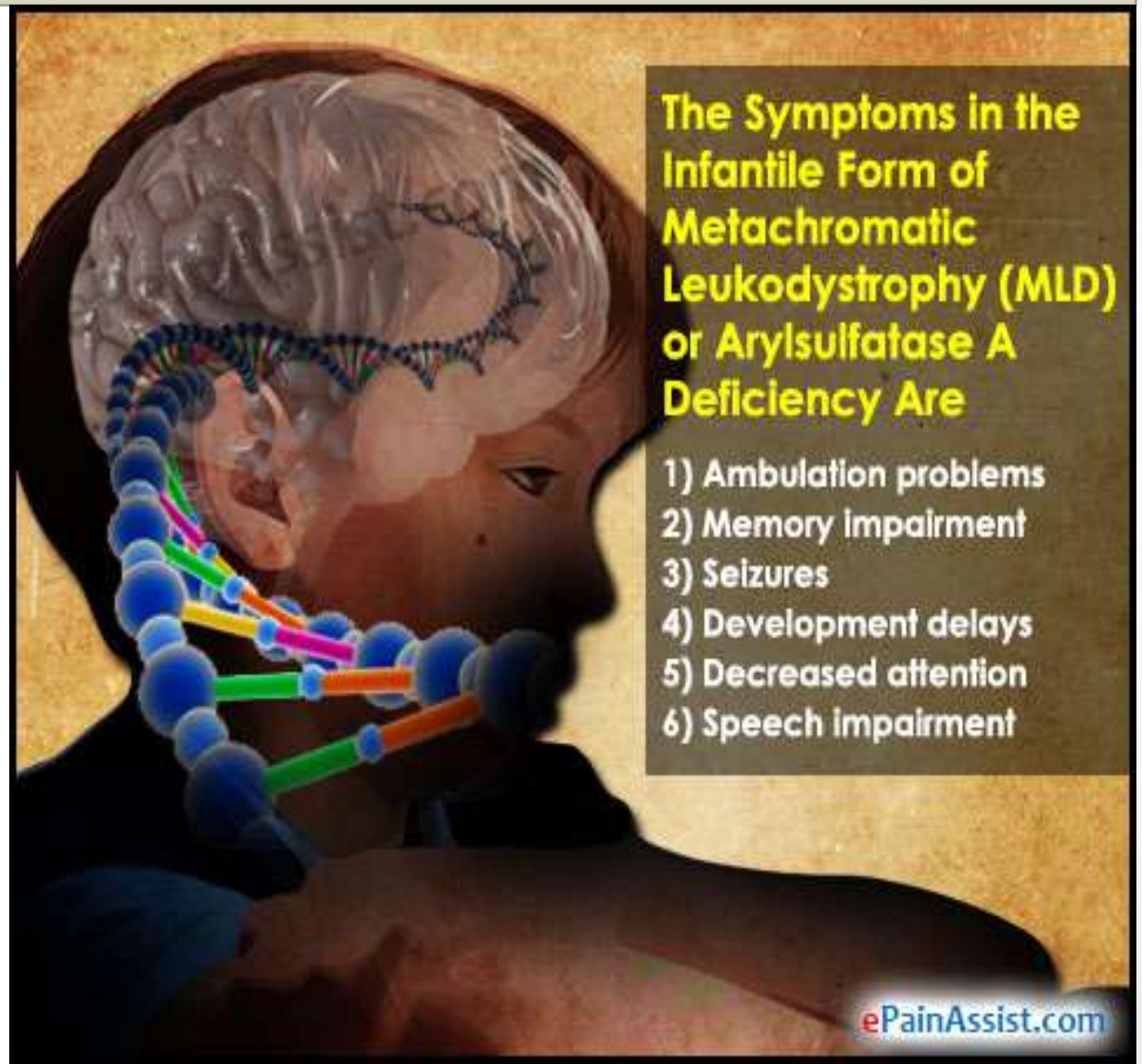
Lipid storage disorders

- The following are some examples:
- GM1 gangliosidosis defect of **β-galactosidase**,
- GM2 gangliosidosis such as **Tay–Sachs disease**, due to **hexosaminidase deficiency**,
- **Gaucher's disease**, due to a deficiency of **β-glucosidase** (glucocerebrosidase),
- **Niemann–Pick** disease, resulting from **sphingomyelinase deficiency**,
- **Fabry's disease**, resulting from **α-galactosidase A deficiency**.
- **Metachromic leucodystrophy**, resulting from **arylsulfatase A deficiency**



Signs& symptoms

- Organomegaly,
- skeletal abnormalities,
- pulmonary infiltration and
- cherry-red macular spot on ophthalmologic examination



Plasma Proteins

2019

Plasma Proteins

- **How is blood plasma different from serum?**
- **What are some of the proteins in blood plasma or serum?**
- **What are the functions of proteins in blood plasma?**

General properties of plasma protein

- Most are synthesized in the liver
  - Exception: γ-globulins – synthesized in plasma cells
- Synthesized as *pre-proteins* on membrane-bound polyribosomes; then they are subjected to posttranslational modifications in ER and Golgi apparatus
- Almost all of them are glycoproteins
  - Exception: albumin
- They have characteristic half-life in the circulation (albumin – 20 days)
- Many of them exhibit polymorphism (immunoglobulins, transferrin…)

the functions of proteins

Blood clotting factors: proteins in coagulation cascade

Immune defense: Immunoglobulins, Complement proteins involved in Inflammatory responses:

Acute phase response proteins: C-reactive protein, alpha-acid glycoprotein (Orosomucoid);

Transport /binding proteins: Albumin, Ceruloplasmin, Haptoglobin, Retinol Binding Protein, Sex Hormone Binding Globulin, Thyroid Hormone Binding Protein, Transferrin.

Anti-proteases: Anti-Chymotrypsin, Anti thrombin, a2-Macroglobulin.

Enzymes: renin, clotting factors, complement proteins

Functions of plasma proteins

Humoral immunity: immunoglobulins

protease inhibitors: a1-antitrypsin

maintenance of all proteins, particularly albumin

Oncotic pressure

Buffering all proteins

Some diagnostic significance of Total Protein

Total Protein in plasma is about 7 –7.5 g/dL

- Plasma proteins includes: Simple Proteins, Mixed or Conjugated Proteins, Glycoproteins and various types of Lipoproteins,
- Changes in amount of Total Protein in plasma are common in some disease conditions;
- **Elevated** amount of Total Protein in plasma may indicate presence of Paraproteins,
- **Decrease** amount of Total Protein in plasma may indicate low level of Albumin

Albumin

- Concentration in plasma: 45 g/l
- ~ 60% of the total plasma protein
- Functions:
  - maintenance of plasma oncotic pressure (values lower than 20 g leads to edema)
  - protein reserve, the source of amino acids
  - transport of:
    - steroid hormones
    - free fatty acids
    - bilirubin
    - drugs (sulfonamides, aspirin)
    - Ca^{2+}
    - Cu^{2+}

the functions of Albumin

Albumin is one of the major plasma proteins; it is synthesized and secreted by the Liver,

- Biological half-life of Albumin in plasma: 20 days
- Significant decrease in amount of Albumin in plasma is usually slow to occur if it is due to reduction in biosynthesis of Albumin,
- Albumin makes the biggest contribution to plasma Oncotic Pressure ,
- **Edema** may occur when plasma Albumin level falls very low,
- Albumin is one of the major binding /transport proteins in blood plasma,

Causes of changes in total plasma

Increase:

Protein synthesis ↑:hyper gamma-globulinemia, para-proteinemia.

Volume of distribution ↓:dehydration

Artefactual: hemo concentration due to stasis of blood during venipuncture

Decrease:

Protein synthesis ↓:malnutrition, mal absorption, liver disease

Volume of distribution ↑: over hydration, increased capillary permeability

Excretion ↑, Catabolism ↑:protein-losing states, catabolic states

some of the Specific Serum/Plasma Proteins

- Measurement of some specific plasma proteins gives useful information for diagnosis and management of some diseases: Examples:

- Transferrin receptors, Ferritin,
- Thyroid Binding Globulin (TBG)
- Sex Hormone Binding Globulin (SHBG),
- Haptoglobin,
- Albumin,
- Globulins,
- C-reactive protein (CRP),
- Immunoglobulins (Ig); etc.

some of the Specific Serum/Plasma Proteins

- Characteristic changes in amount of certain plasma proteins are seen after Surgery or Trauma, or during Infection or Tumor growth:

Proteins involved are called Acute Phase Proteins;

•**Acute Phase Protein response** leads to greatly increased De Novo biosynthesis (mainly in Liver) of some plasma proteins along with decease in levels of other proteins in plasma,

•**Response is stimulated by release of Cytokines**: Interleukin-1, Interleukin-6and Tumor necrosis factor (TNF)and increased plasma [Cortisol] and [Glucagon]

some of the Specific Serum/Plasma Proteins

- Acute Phase Protein response is an adaptive response to diseases;

Example:

•Increases in plasma levels of CRP and Complement will contain and eliminate infection,

•Increased Coagulation Factors will aid and prevent excess blood loss,

•Protease Inhibitors will prevent the spread of tissues necrosis when damaged cells at the site of injury release Lysosomal enzymes,

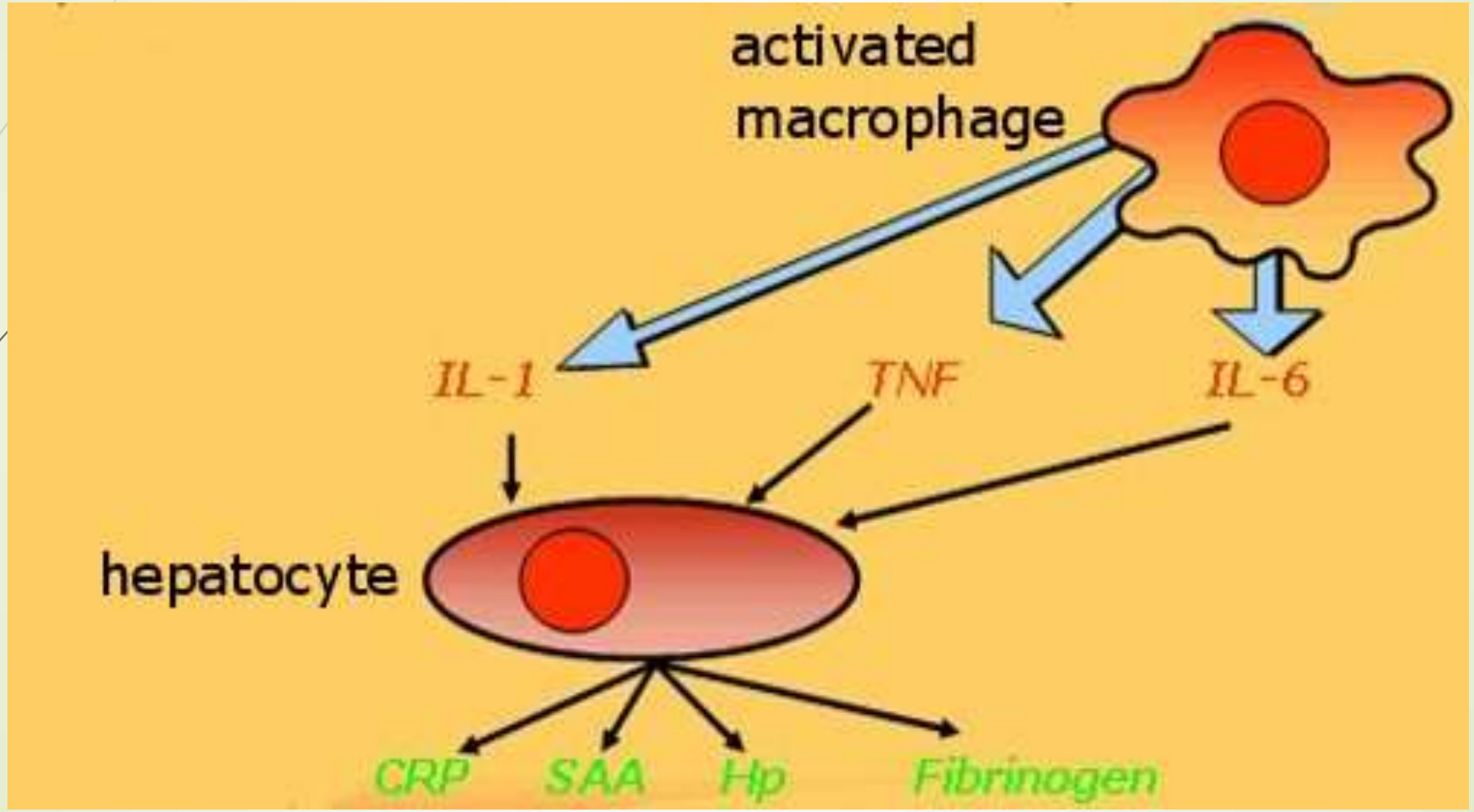
•**Clinically some Acute Phase proteins** are used to monitor progress of some disease condition or its response to treatment;

Acute phase reactants (APRs)

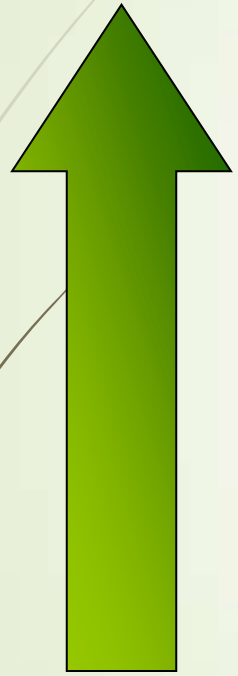
- Their levels change during acute inflammatory response
- Cause conditions where there is:
  - ✓ the destruction of cells
  - ✓ the reversible cell damage and subsequent repair
  - ✓ the metabolic activation of certain cells (immune cells)

- APRs concentration changes in:
  - infection
  - surgery
  - injury
  - cancer

Acute phase reactant response



Types of APRs:



Positive:

C-reactive protein:
~1000-fold increase!

α_1-antitrypsin

fibrinogen

haptoglobin (HP)

C3, C4

serum amyloid A (SAA)

Negative:

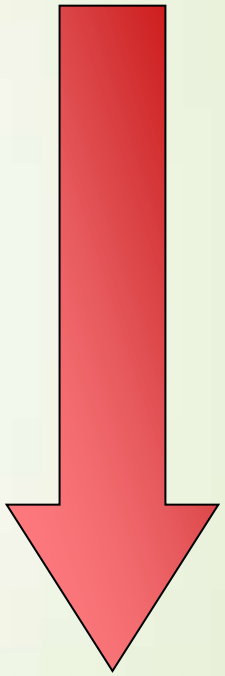
albumin

transferrin

antithrombin

transcortin

retinol binding protein



The importance of positive acute phase reactants

- Components of the immune response
  - C-reactive protein, complement components (C3 a C4),TNF-α, Il-1, Il-6
- Protection against collateral tissue damage
  - scavergers of ROS and protein stabilizing transition metals and their complexes
    - haptoglobin
    - hemopexin
    - feritin
    - ceruloplasmin
  - *Inhibitors of proteases*
    - α_1-antitrypsin
    - α_1-antichymotrypsin
    - α_2-macroglobulin

The importance of positive acute phase reactants

- Transport of waste products produced during inflammation :
  - hemoglobin
  - hemopexin
  - serum amyloid A (SAA)
- Coagulation factors and proteins involved in tissue regeneration :
  - fibrinogen
  - prothrombin
  - factor VIII
  - von Willebrandt factor
  - plasminogen

The importance of negative acute phase reactants

- The criterion for determining inflammation (decrease inflammation)
  - transcortin (corticoid binding protein)
- The criterion for protein synthesis in the liver

Fractions of plasma proteins

Fraction	*Rel. amount (%)*	*c (g/l)*
Albumins: albumin pre-albumin (transthyretin)	52 – 58	34 – 50
α_1-globulins: thyroxin-binding globulin, transcortin, α_1-acid glycoprotein, α_1-antitrypsin, α_1-lipoprotein (HDL), α_1-fetoprotein	2,4 – 4,4	2-4
α_2-globulins: haptoglobin, macroglobulin, ceruloplasmin	6,1 – 10,1	5 – 9
β-globulins: transferrin, hemopexin, lipoprotein (LDL), fibrinogen, C-reactive protein, C3 and C4 components of the complement system	8,5 – 14,5	6 – 11
γ-globulins: IgG, IgM, IgA, IgD, IgE	10 – 21	8 – 15

Electrophoresis may be used to study protein abnormalities;

Serum is a **better choice** for Electrophoresis, because the Fibrinogen of Plasma gives a discrete band, which can easily be mistaken for Paraproteins

General pattern of electrophoresis result

- Shows order of migration along **Horizontal Axis** with proteins of highest mobility closest to Anode,
- Height of the band along the **Vertical Axis** shows the protein concentration,
- Location of some major proteins are indicated underneath their Electrophoretic mobility peaks

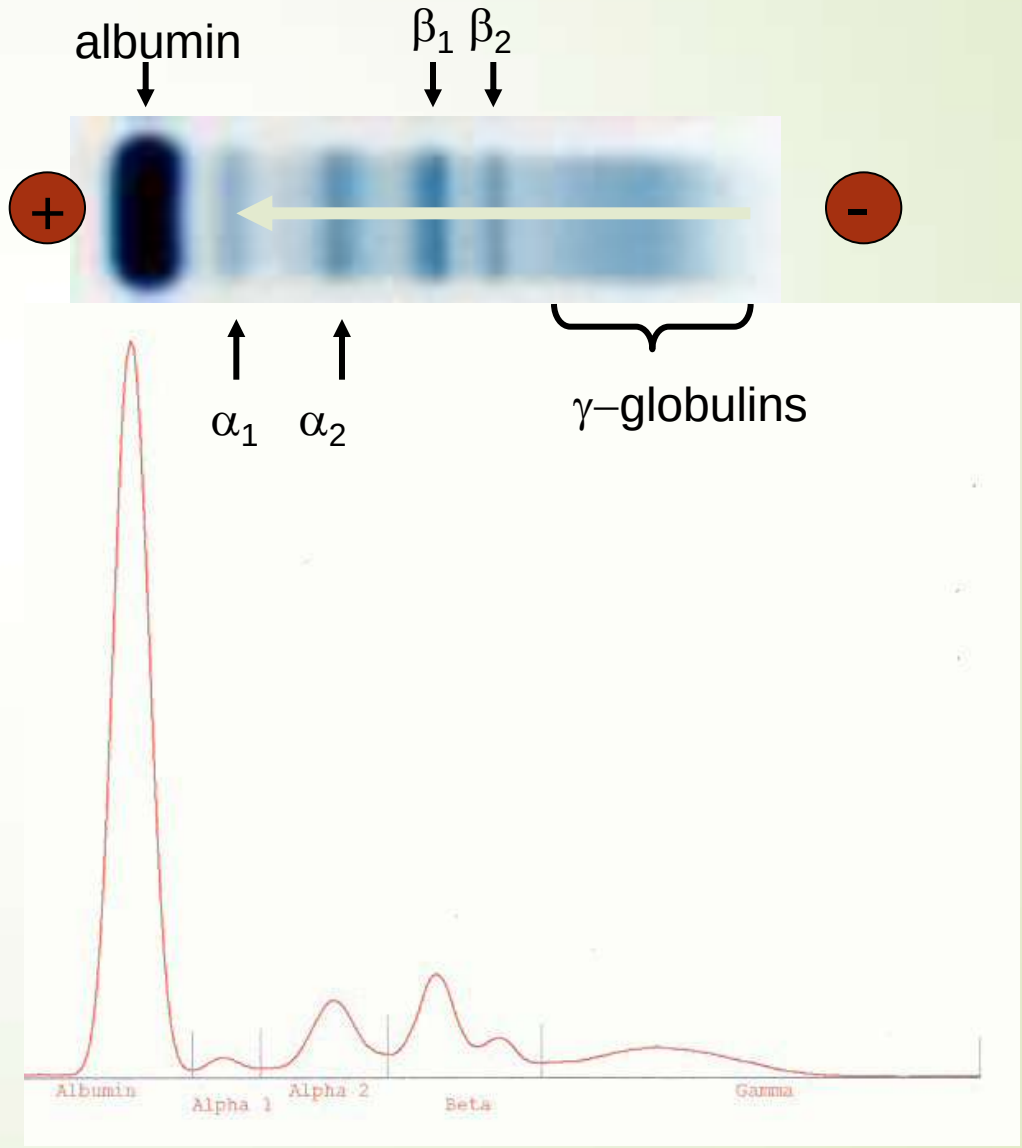
Electrophoresis can also show gross deficiency or excess of Immunoglobulins and whether Paraproteins are present

Quantitative measure of each protein class may be obtained by scanning Electrophoretic strip

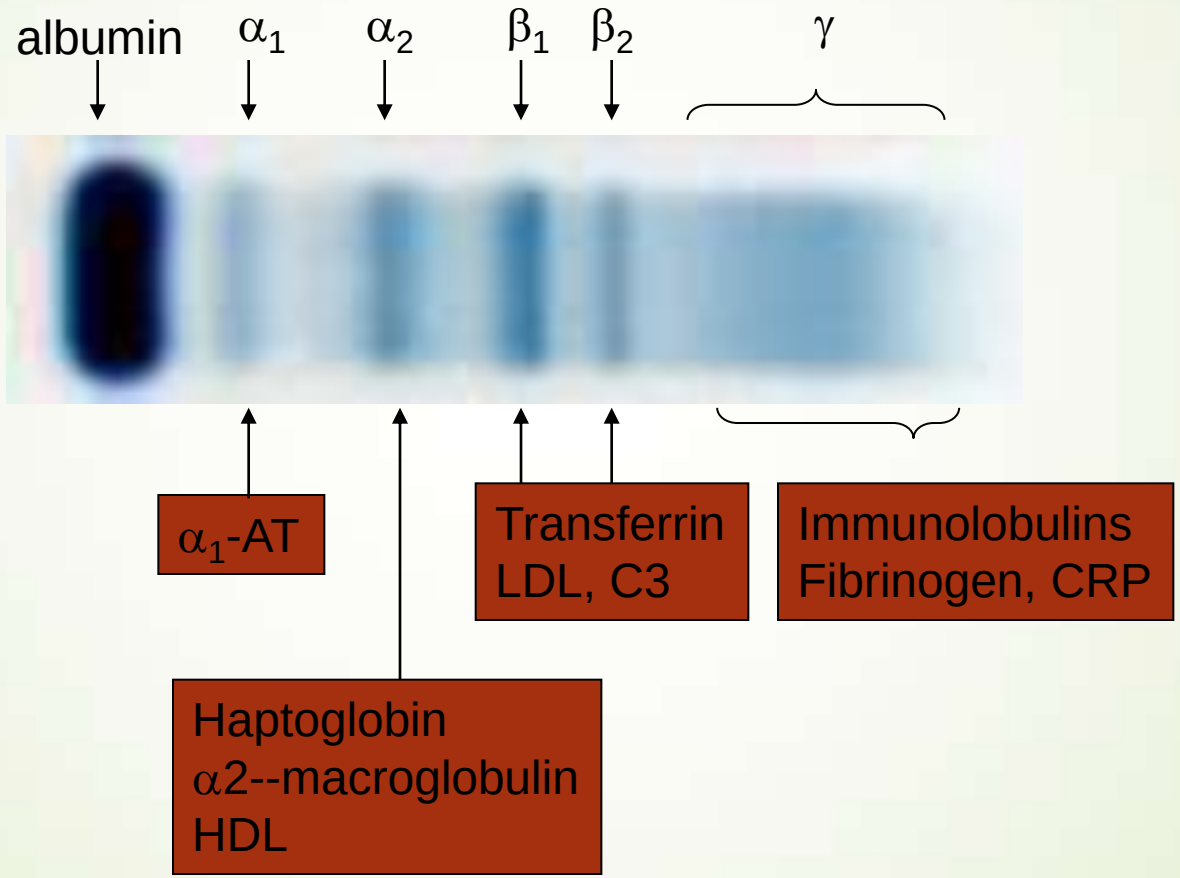
Types of plasma proteins

Electrophoresis of plasma proteins

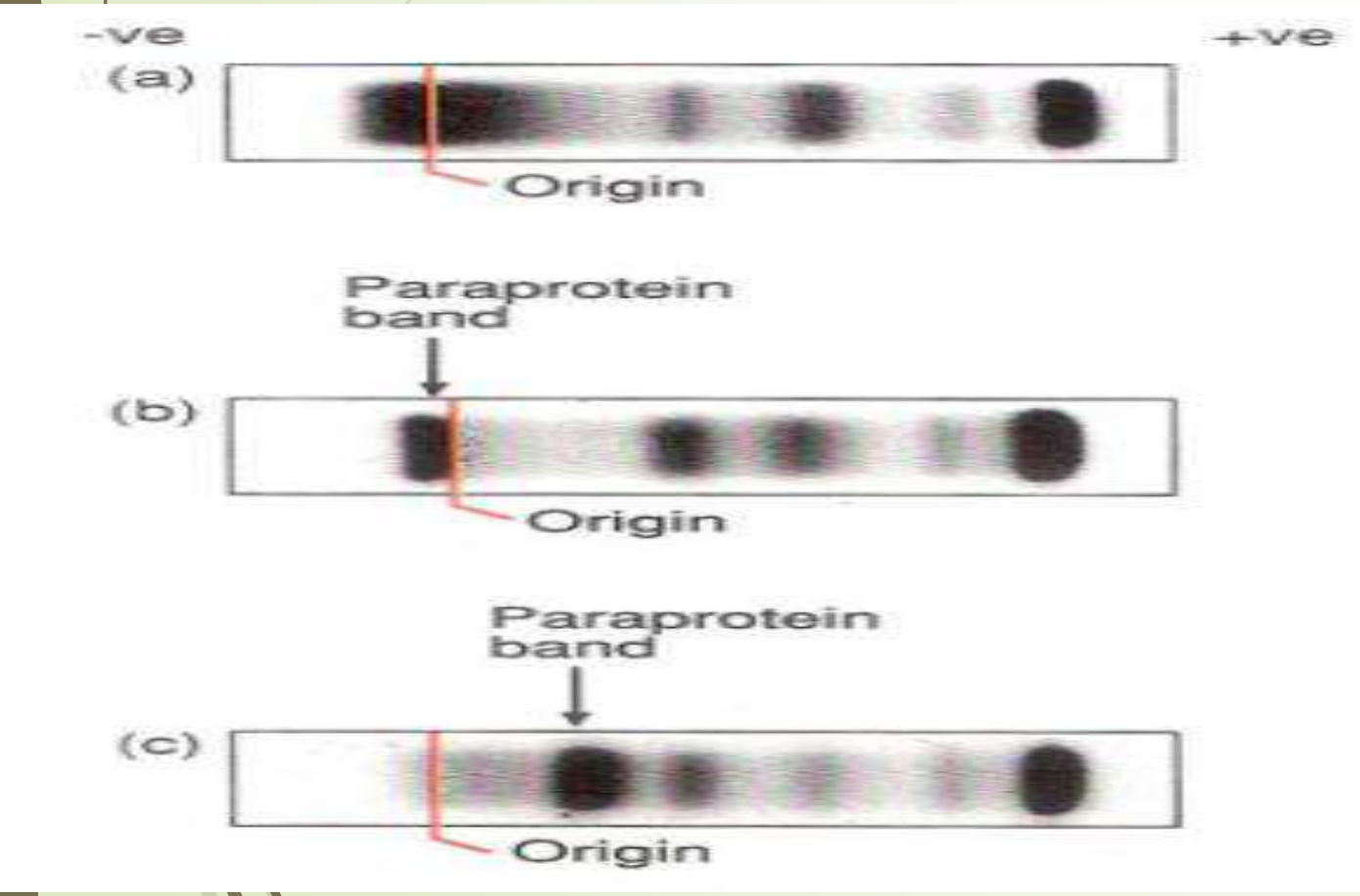
Proteins move in an electric field according to their charge and size.

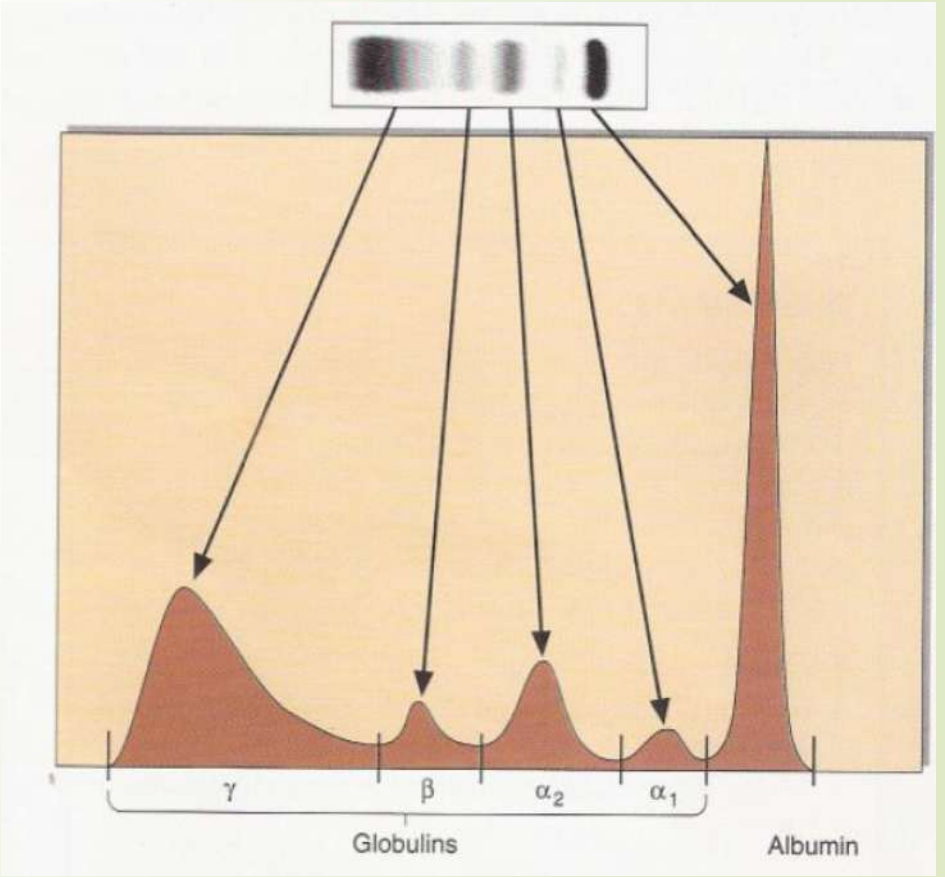


The main components of globulin



Electrophoresis of Serum proteins: (a) Normal pattern, (b) Presence of Paraproteins, (c) Presence of Paraproteins





Immunoglobulins

- Immunoglobulins are a group of structurally related proteins that function as Antibodies;

•Immunoglobulins are produced by cells of the Lympho-reticular System,

•Immunoglobulins are also produced by Plasma Cells, which are B-lymphocytes transformed after exposure to foreign (occasionally an endogenous) Antigen

IMMUNOGEN is a molecule that can generate an Immune response (cellular or Humoral).

ANTIGEN is a molecule that reacts with Antigen Receptors, irrespective of its ability to generate an Immune Response,

- Antigen may, or may not be an Immunogen,

HAPTEN is a small molecule that is able to react with preformed Antibodies

Hapten has Antigenicity, but is not capable to stimulate specific Immune Response (is not Immunogenic)

Haptens are only Immunogenic when coupled to a large protein called a carrier

All Immunogens are therefore Antigenic **but** not **all Antigens** are Immunogenic

- **Epitopes or Antigenic determinants**:

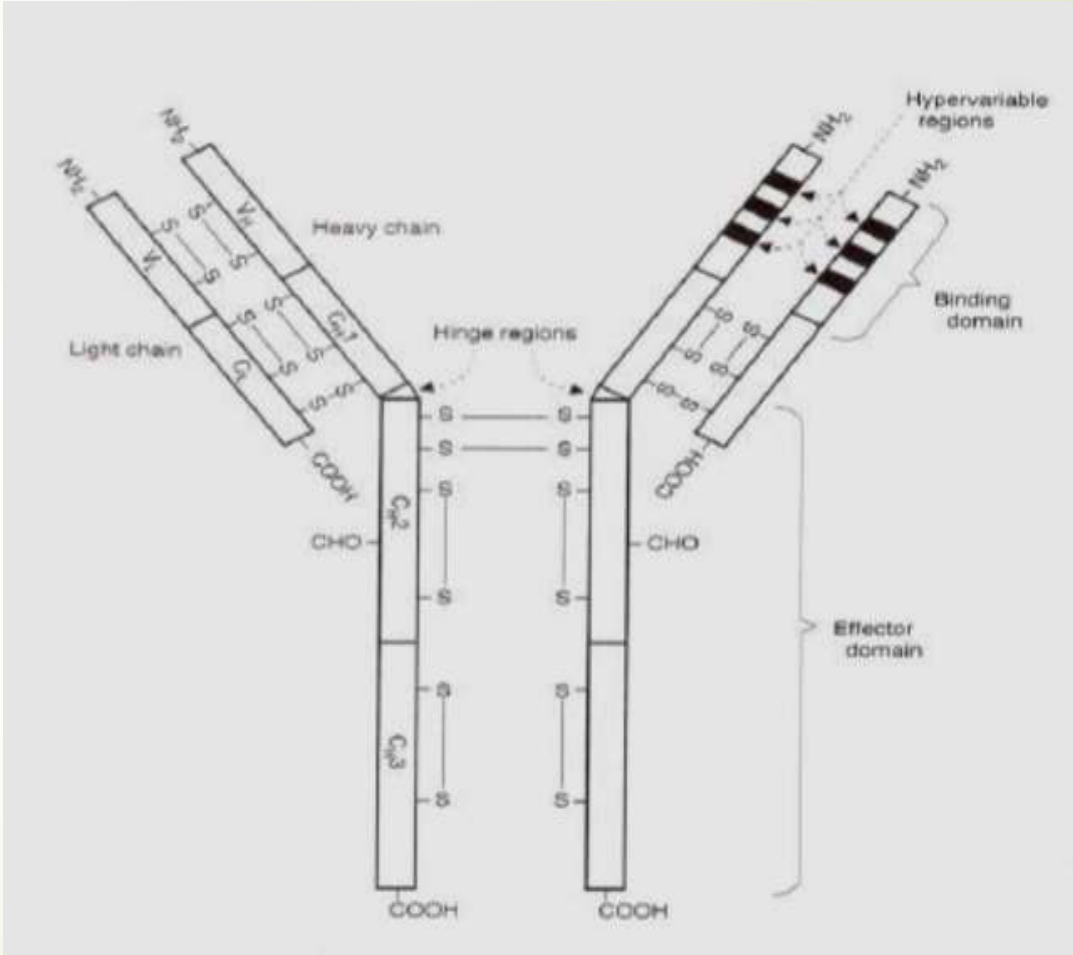
•**Antigen Receptors** on Lymphocytes recognize discrete sites on an Antigen called Epitopes or Antigenic Determinants

•**Antigen** recognition by B-cells and T-cells is fundamentally different and does not involve the same Epitopes

Immunoglobulin

Basic structure of Immunoglobulin (Ig):

- 2 Identical "Heavy" Polypeptide Chains, and
- 2 Identical "Light" Polypeptide Chains;
- Both Chains have Inter-chain and Intra-chain Disulfide (S-S) Bonds and Non-covalent Interactions



Immunoglobulin

- Two types of "Light" Polypeptide Chains:

•Kappa "Light" Chains

•Lambda "Light" Chains

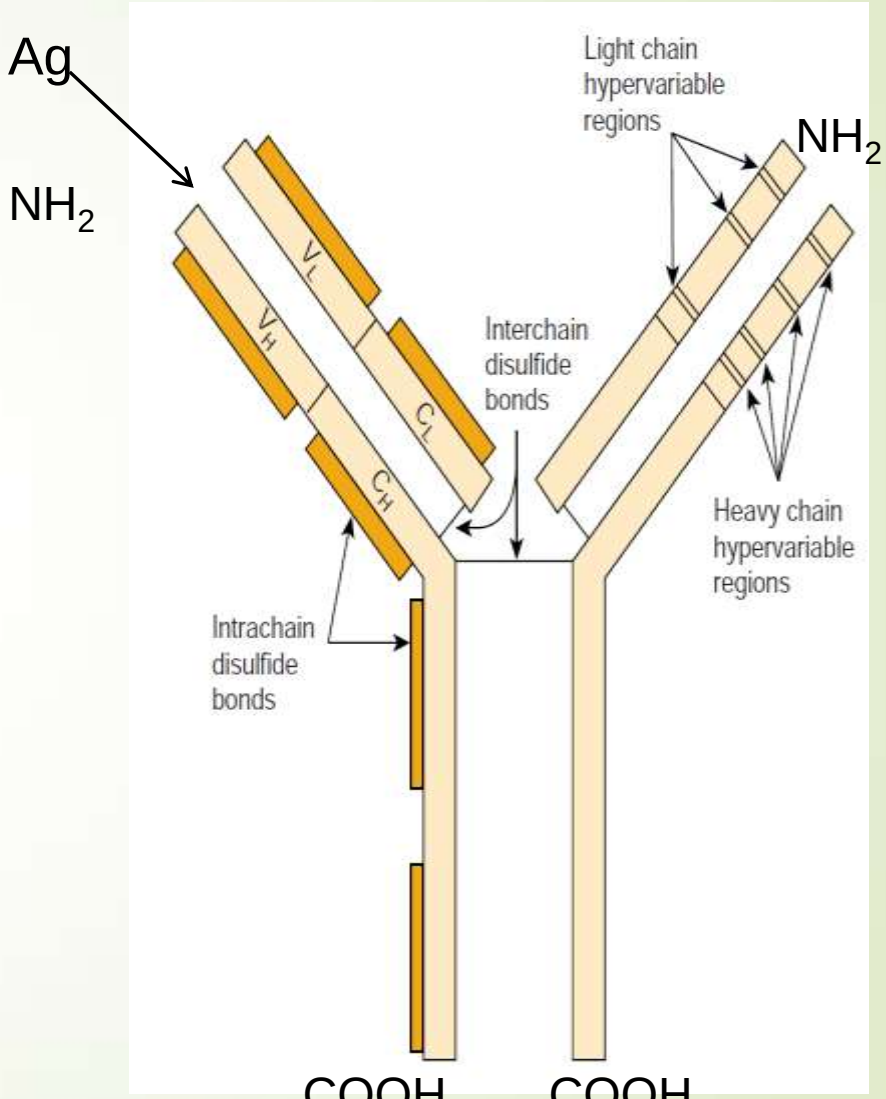
- Five principal types of Heavy Polypeptide Chains:

IgA, IgG, IgD, IgE, IgM

Alpha, Gamma, Delta, Epsilon, Mu

Immunoglobulins

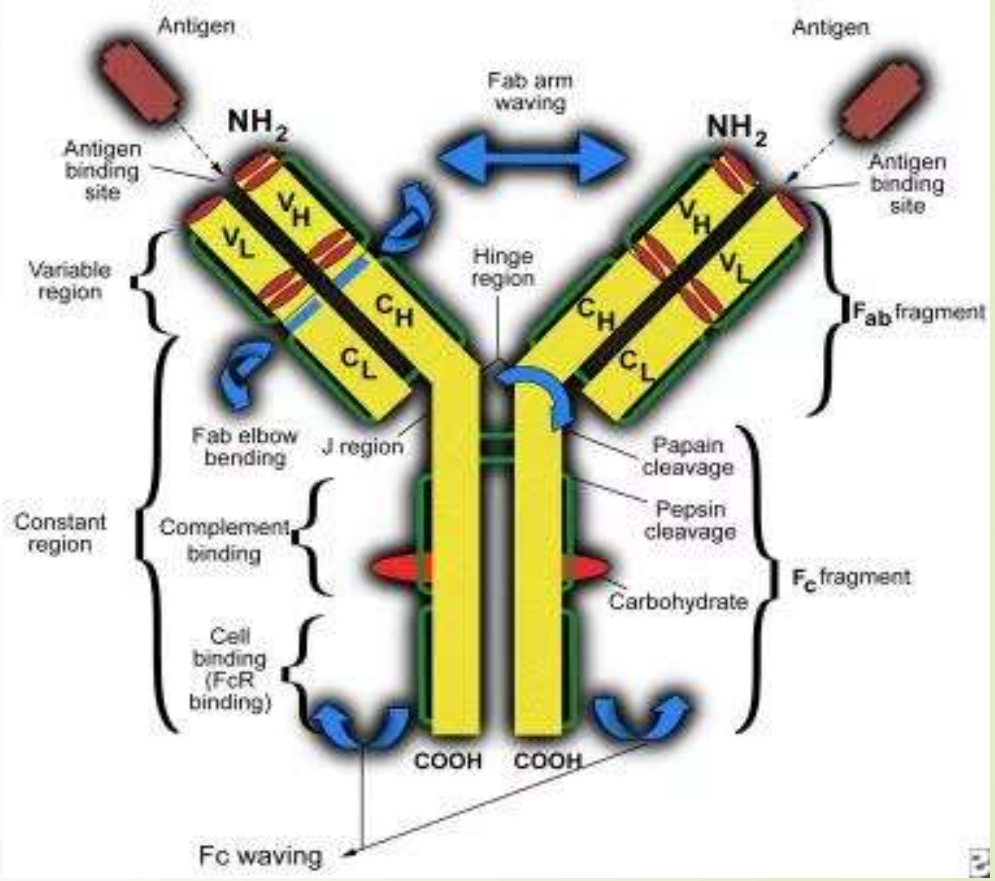
- Antibodies produced by B cells in response to antigen stimulation of the organism
- React specifically with antigenic determinants
- Structure:
  - consist of a minimum of 4 polypeptide chains - 2 heavy (H) a 2 light (L) linked by disulfide bridges
    - light chains contain constant (C) and variable (V) region



IgG

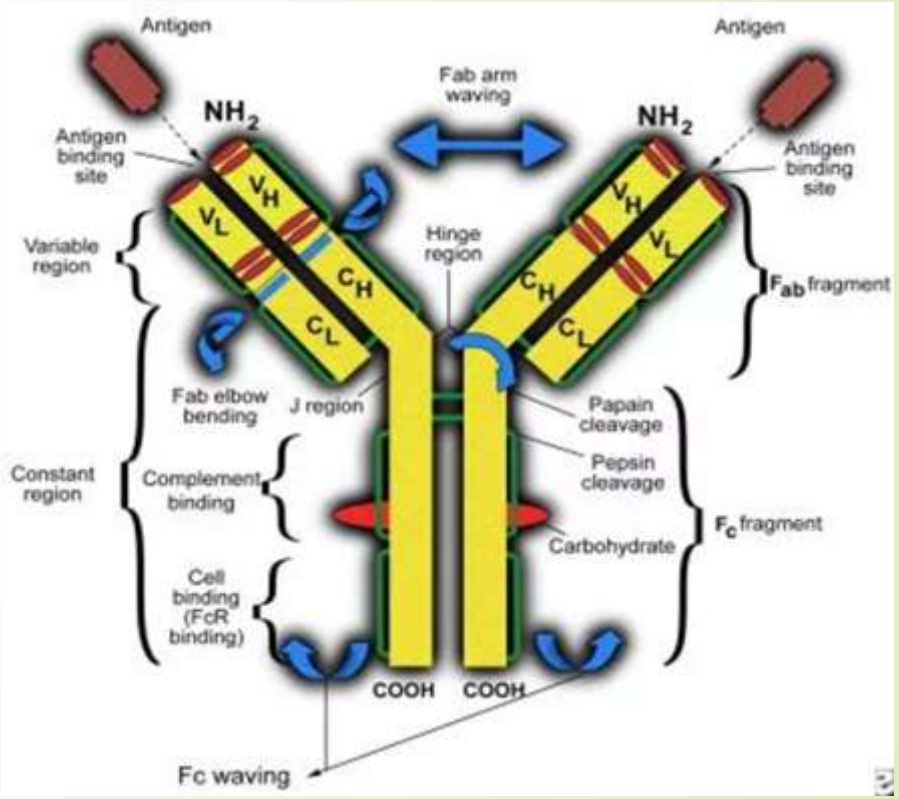
Action of Papain on IgG

- Papain a protease enzyme acts on the **Hinge region**(in front of inter chain S-S bonds) in IgG
- Hydrolysis of Ig G by **Papain** gives **3 components**:
- Two Identical Fab(Fragment antigen-binding) fragments,
- One Fc(Fragment-crystalizable) fragment



Action of Pepsin on IgG:

- Pepsin a protease enzyme acts on **Hinge region** (behind the inter chain S-S bonds) in IgG,
- Hydrolysis of IgG by **Pepsin** produces:
- A single divalent F(ab')2 and
- A p Fc' fragment



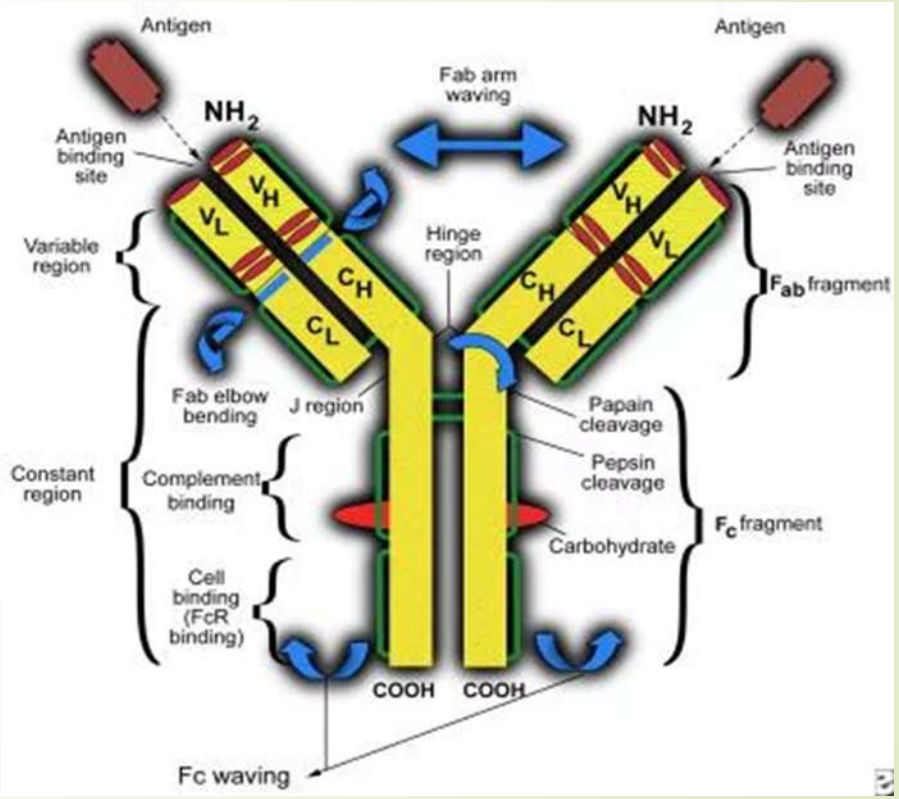
V regions: Amino-terminal portions of Heavy and Light chains show considerable variability in Amino Acid composition;

•Hyper-Variable Regions or Complementarity-Determining Regions: Three areas in V regions of Light and Heavy chains that have remarkably diverse amino acid sequences;

•C region: Parts of Heavy and Light chains that are relatively Constant in terms of Amino Acid composition;

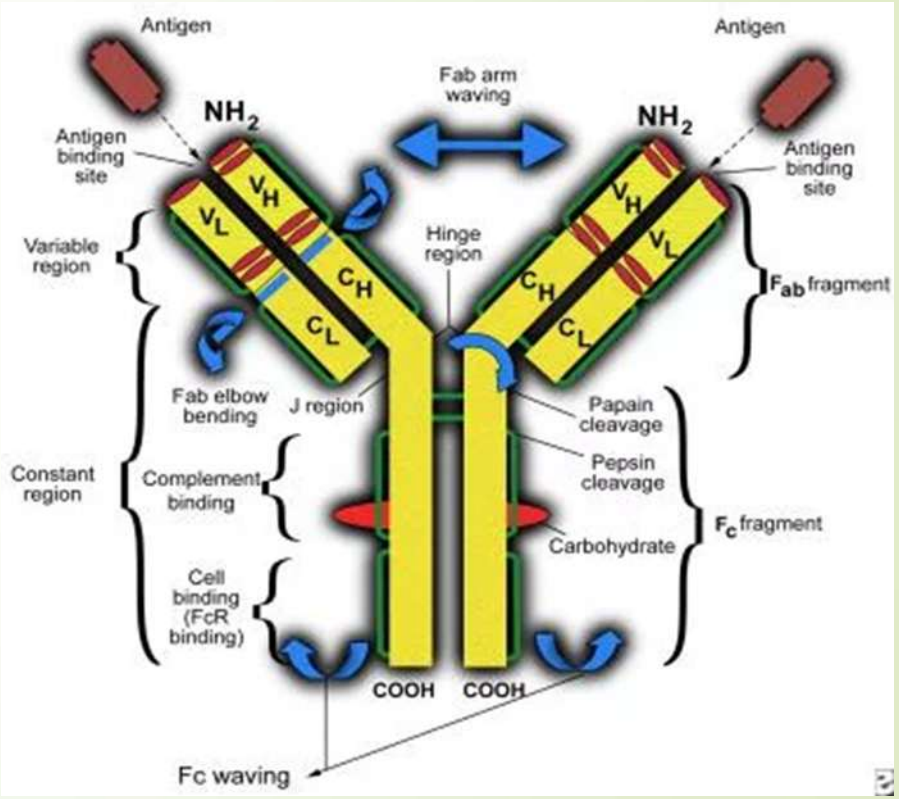
•Light chains contain One Variable Domain (VL) and One Constant Domain (CL);

•Heavy chains contain One Variable Domain (VH) and 3 or 4 Constant Domains designated CH 1 –3 or CH 1 –4) accordingly



Ig molecules contain two functional areas:

- Fab, or Variable end –is the area that recognizes and binds to Antigens;
- Fc end –is responsible for interaction with other components of Immune system, e.g., Complement and T helper cells;
- Hyper-variable Loops form Antigen-Binding Site of an Immunoglobulin molecule, i.e.,
- Each Hyper-variable Loop contributes to the Antigenic Specific or Complementarity of the binding site for Antigen;



- Various Types of Immunoglobulins have different Tertiary structure and Functions;

Major Immunoglobulins in plasma are:

•IgG: neutralizes toxins, activates complement, capable of crossing Feto-placental barrier;

•IgA: contains J chain and secretary component, part of defense against viral and bacterial infections;

•IgM: usually first to be made in immune response, contains J chain, in presence of complement are very effective in producing Lysis of cells;

Ig may be increased non-specifically in a wide variety of Infections and in Autoimmune diseases

- Increase biosynthesis of Ig may be cause by several Cell Lines, each producing specific type of Immunoglobulins (Hyper-Gamma-Globulinemia)
- Such response is said to be "Polyclonal" and results in diffuse increase in protein mass throughout the Gamma Globulin region;
- Appears as broad band during Electrophoresis of Serum protein

Increase biosynthesis of Ig may be cause by a Single Clone of cells making Identical Ig;

- Such is said to be "Monoclonal"
- Ig production may increase, becomes large enough to be observed as a single discrete band on electrophoresis of the serum,
- Such single discrete band may be due to increase in Intact Immunoglobulin or fragments called **Paraproteins**

Paraproteins (Monoclonal components): discrete Ig bands, seen on electrophoresis of Serum,

- Paraproteins are due to production of Single type of Ig or Ig fragments (Light-chain or Heavy-chain fragments) by a Single clone of B cells,
- Paraproteins may arise from any of the Ig classes
- Detection of Paraproteinin blood or urine requires further investigation to determine if the Paraproteinemiais caused by Benign or Malignant condition

Benign Paraproteinemia may occur transiently during acute infection and in autoimmune disease due to Antigen stimulation,

Paraproteins are found in malignant conditions such as:

- Multiple Myeloma,
- Macroglobulinemia,
- Heavy chain diseases,

Monoclonal Light Chains are produced in excess of Heavy chains in about 50% of cases of Myeloma, and in about 15% of cases only Light chains are found;

- These light chains are small enough to spill into urine, they are known as Bence-Jones Protein,
- Serum electrophoresis may not show the presence of light chains,
- Urine electrophoresis after concentration may be required to demonstrate the Paraproteins

Myeloma

Myeloma is characterized by Bony Metastases,

- Bone pain is often the presenting symptom,
- In the face of increasing synthesis of abnormal Immunoglobulins, other bone marrow function is reduced, and there is a decline in Red and White cell and Platelet formation and decreased production of normal Immunoglobulins,
- Anemia and susceptibility to infection are the usual consequences;

Pathologic changes of plasma proteins

- Dysproteinemia: total plasma protein concentration is normal, but the normal ratio of its components is changed example: acute inflammation, chronic inflammation.
- Defect dysproteinemia: total absence of a certain plasma protein example: lack of albumin, lack of alfa-1 antitrypsin, lack of ceruloplasmin
- Paraproteinemia: There is a protein in the plasma, which cannot be detected under normal conditions example: monoclonal gammopathy

Causes of Albumin Deficiency

- Liver diseases (cirrhosis) – decrease in the ratio of albumin to globulins
- Protein malnutrition
- Excessive excretion by kidneys (renal disease)
- Mutation causing analbuminemia (affects splicing)

α_1-antitrypsin

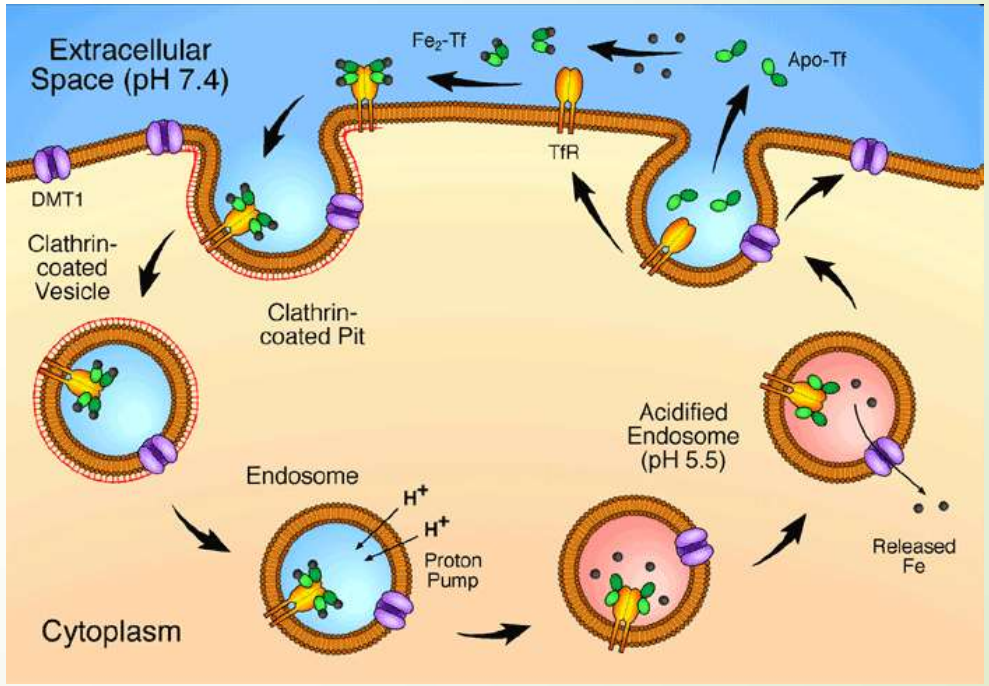
- Main globulin of α_1 fraction (90 %)
- is synthesized in the liver in hepatocytes and macrophages
- glycoprotein, highly polymorphous (≈75 forms)
- Function:
  - Main plasma inhibitor of serine proteases (trypsin, elastase...)
  - during the acute phase increases ⇒ inhibition of degradation of connective tissue by elastase
  - deficiency ⇒ proteolytic lung damage (emphysema)

Transferrin

- Transferrin is a β-globulin
- It binds free iron in serum
- Normally it is about one third saturated with iron
- Transferrin levels are decreased in:
  - *liver disease (cirrhosis)*
  - *Chronic infections*
  - *Nephrosis*
  - *Congenital atransferrinaemia*
- Increased serum transferrin levels occur during increased transferrin synthesis caused as a result of iron deficiency anemia

Receptor-mediated transferrin endocytosis

- Ferro-transferrin binds to the receptors on the cell surface → the complex is internalized into an endosome
- In endosomes, iron dissociates from transferrin (enabled by low pH & Fe^{3+} → Fe^{2+} reduction) and enters cytoplasm
- Iron is delivered to intracellular sites or bound to ferritin (Fe^{2+} → Fe^{3+} oxidation and Fe^{3+} storage)
- Apotransferrin, associated with the receptor, returns to the membrane, dissociates from the receptor and re-enters plasma.



Transferrin

- Free Fe^{2+} ions are toxic for organism – catalyses Fenton reaction (formation of highly toxic $^{\cdot}OH$ radical)

  $H_2O_2 + Fe^{2+} \rightarrow OH^- + {}^{\cdot}OH + Fe^{3+}$

- Transferrin with other plasma proteins that bind iron or heme, acts as an antioxidant (prevents ROS)
- Causes of decline in transferrin :
  - burns, infections, malignant processes and liver and kidney diseases
- Cause of relative transferrin excess:
  Iron-deficiency anemia

Ferritin

- Intracellular protein; only small portion in plasma
- 24 subunits surround 3000 - 4500 ions of Fe^{3+}
- Function: stores iron that can be called upon for use when needed
- Primary hemochromatosis – genetic disorder characterized by increased absorption of iron from the intestine $\Rightarrow$ accumulated iron damages organs such as the liver, skin, heart, and pancreas. Concentration of ferritin is elevated.

Cerruloplasmin

- Conc. in plasma: 300 mg/l

- Functions:
  - carries 90% of copper in plasma (copper – cofactor for a variety of enzymes)

    1 molecule binds 6 atoms of copper

    binds copper more tightly than albumin that carries other 10% of plasma copper ⇒ albumin may be more important in copper transport (donates copper to tissues more readily)

Haptoglobin (Hp)

- α_2- globulin, tetramer $\alpha_2\beta_2$ chains
- Exists in 3 polymorphic forms
- Functions:
  - binds free hemoglobin and delivers it to the reticulo-endothelial cells
  - complex Hb- Hp is too large to pass through glomerulus $\Rightarrow$ prevention of loss of free Hb (and Fe)

Free Hb passes through glomerulus, enters tubules and tends to precipitate there in $\Rightarrow$ kidney damage

Causes of Hp **increase**

- Hp belongs to APRs ⇒
  - inflammation, infection
  - injury
  - malignancies

Causes of Hp decrease

- Hemolytic anemia:
  - half-life of Hp = 5 days X of complex Hp-Hb = 90 min (the complex is being rapidly removed from plasma) ⇒ Hp levels fall when Hb is constantly being released from red blood cells (as in hemolytic anemias)

Plasma proteins as antioxidants

Transferrin

Ferritin

Ceruloplasmin

Haptoglobin

Hemopexin (binds heme and transfers it to the liver)

act as antioxidants:

remove Fe^{2+} and thus prevent the Fenton reaction:

$$H_2O_2 + Fe^{2+} \rightarrow HO^{\bullet} + OH^{-} + Fe^{3+}$$

C-reactive protein (CRP)

- Belongs to β_2-globulin, the levels of which rise in response to inflammation
- Acute-phase reactant
- Its physiological role is to bind to phosphocholine expressed on the surface of dead or dying cells (and some types of bacteria)
- plasma concentration levels of CRP rapidly increase within 2 hours of acute insult, reaching a peak at 48 hours (bacterial, viral, fungal infection, rheumatic diseases, malignity, tissue necrosis)

Fibrinogen

- Glycoprotein, belongs to β_2-globulins (Mr 340 000)
- Concentration in plasma - 1.5 – 4.5 g/l
- component of the coagulation cascade – fibrin precursor

- Acute-phase reactant ⇒ ↑ acute inflammation

Plasma enzymes

Plasma specific enzymes:

- cholinesterase,
- super-oxid dismutase,
- lecithin-cholesterol acyl transferase,
- Serin proteases – inactive zymogens of coagulation factors and factors of fibrinolysis (factor II - prothrombin, factor VII, IX, XIII) and complement system components, non-specific immune system (components C1 – C9).

Plasma enzymes (cont.)

Enzyma name	**abbreviation**	**Causes leading to increased levels**
Alanine aminotransferase	ALT	liver and biliary tract disease pancreatic disease decompensated heart defects
Aspartate aminotransferase	AST	liver diseases myocardium damage disease of skeletal muscle and myocardium
Alkaline phosphatase	ALP	liver and biliary tract disease bone diseases
Creatin kinase	CK	disease of skeletal muscle and myocardium
Lactate dehydrogenase	LD_{1-5}	Myocardium disease (LD_1, LD_2) and muscle disease hepatopathy
γ-glutamyl transferasa	GMT	liver and biliary tract disease and pancreatic disease

PITUITARY HORMONES

2019

ANTERIOR PITUITARY HORMONES

- Hormones---- **Tropic**
- Hormones---- **Direct effectors**

Anterior pituitary hormones

PITUITARY HORMONE	TARGET GLAND	STRUCTURE	FEEDBACK HORMONE
Luteinizing hormone (LH)	Gonad (tropic)	Dimeric glycoprotein	Sex steroids (E_2/T)
Follicle-stimulating hormone (FSH)	Gonad (tropic)	Dimeric glycoprotein	Inhibin
Thyroid-stimulating hormone (TSH)	Thyroid (tropic)	Dimeric glycoprotein	Thyroid hormones (T_4/T_3)
Adrenocorticotropin hormone (ACTH)	Adrenal (tropic)	Single peptide derived from POMC	Cortisol
Growth hormone	Multiple (direct effector)	Single peptide	Insulin-like growth factor (IGF-I)
Prolactin	Breast (direct effector)	Single peptide	Unknown

T_4, thyroxine; T_3, triiodothyronine; E_2, estradiol; T, testosterone.

PITUITARY TUMORS

1-Prolactin-secreting pituitary tumors are the most common, followed by **nonfunctioning or null cell tumors**,

2- Tumors that secrete **GH, gonadotropins, ACTH, or TSH** account for the remainder.

- **Physiologic enlargement** of the pituitary can be seen during **puberty and pregnancy.** The enlargement seen during pregnancy is due to **lactotroph hyperplasia**.

- **Thyrotroph or gonadotroph hyperplasia** can also be seen in longstanding primary thyroidal or gonadal failure.

GROWTH HORMONE

- **Growth hormone** (*somatotropin)* is structurally related to **prolactin and human placental lactogen**.
- A single peptide with **2 intramolecular S-S**, it belongs to the **direct effector** class of anterior pituitary hormones. The **somatotrophs**, pituitary cells that produce **GH**, cover over ***one third of normal pituitary weight***.
- **Release of somatotropin** from the pituitary is **stimulated** by the hypothalamic peptide **growth hormone-releasing hormone (GHRH)**; somatotropin's secretion is **inhibited** by **somatostatin (SS).**

GROWTH HORMONE

- GH is secreted **in pulses**, with an average inter pulse interval of **2–3 hrs**, with the **most reproducible peak** occurring at the **onset of sleep**.

- **Between these pulses**, the level of GH may fall below the detectable limit, resulting in the clinical evaluation of GH deficiency.

Modifier of GH secretion

STIMULATE GROWTH HORMONE SECRETION	INHIBIT GROWTH HORMONE SECRETION
Sleep	Glucose loading
Exercise	β-Agonists (e.g., epinephrine)
Physiologic stress	α-Blockers (e.g., phentolamine)
Amino acids (e.g., arginine)	Emotional/psychogenic stress
Hypoglycemia	Nutritional deficiencies
Sex steroids (e.g., estradiol)	Insulin deficiency
α-Agonists (e.g., norepinephrine)	Thyroxine deficiency
β-Blockers (e.g., propranolol)	

Actions of Growth Hormone

- it is considered an **amphibolic hormone** because it directly influences both **anabolic and catabolic** processes.
- **One major effect of GH** is that it allows an individual to effectively transition from a fed state to a fasting state without experiencing **a shortage of substrates** required for normal intracellular oxidation.
- GH directly **antagonizes** the effect of insulin on **glucose metabolism**, promotes **hepatic gluconeogenesis**, and stimulates **lipolysis**.

GH

- The anabolic effects of GH are reflected by enhanced **protein synthesis** in skeletal muscle and other tissues.
- This is translated into a **positive nitrogen balance** and **phosphate retention**. GH has **direct effects** on many tissues.
- **Indirect effects** that are mediated by factors that were initially called ***somatomedins***. that there was **more than one somatomedin**, and, because of their structural **homology to proinsulin**, the nomenclature shifted to **insulin-like growth factor (IGF)**.

GH

- **Somatomedin C= IGF-I;** the major growth factor induced by GH. IGFs also have cell surface receptors that are different from insulin.
- **GH stimulates** the production of **IGF-I from the liver** and, as a result, IGF-I becomes a biologic amplifier of GH levels. IGFs are complexes to specific serum binding proteins **(IGFBP-III).**
- **The levels of IGFBP III** are positively correlated with **IGF-I levels** and, as a result, **GH levels**.

Testing

- A single, random measurement of GH is rarely diagnostic.
- Circulating levels of IGF-I, IGFBP-III reasonably integrate the peaks of GH secretion, and elevated levels of both are consistent with a sustained excess of GH.
- **Hepatomas** can be associated with **high levels of IGF-I**, and levels of **IGFBP-III may be inappropriately normal** in some people with **active acromegaly.**
- **Low IGF-I levels** may reflect inadequate production of **GH**; **poorly controlled diabetes**, **malnutrition**, or other **chronic illnesses**.

OGTT

- Definitive testing for determining the autonomous production of GH relies upon the normal suppress ability of GH.
- Patients with acromegaly, GH levels fail to suppress and may even paradoxically rise glucose blood level.
- Testing patients for **suspected GH deficiency** is more complicated.

Test

- Combination **infusions of GHRH and L-arginine** or an **infusion of L-arginine coupled with oral L-DOPA** are the most widely used.
- If GH levels rise **above 3–5 ng/mL**, it is unlikely that the patient **is GH deficient.**
-

Acromegaly

- Acromegaly results from pathologic or autonomous GH excess and, in the vast majority of patients, is a result of a **pituitary tumor**.
- The **ectopic production of GHRH** and although very interesting or helpful, the ectopic production of GHRH or GH remains rare.
- **If a GH-producing tumor occurs before epiphysial closure**, the patient develops **gigantism** and may grow to an **impressive height**; otherwise, the patient develops classical, but insidious, features of bony and soft tissue overgrowth.

Acromegaly features

- progressive **enlargement of the hands and feet** as well as growth of **facial bones**, including the **mandible and bones of the skull.**
- In advanced cases, the patient may develop significant **gaps between their teeth.**
- Diffuse **overgrowth of the ends of long bones or the spine** can produce a debilitating form of arthritis.
- Because **GH is an insulin antagonist**, glucose intolerance or plain diabetes can occur.
- HT**; accelerated atherosclerosis**; and **proximal muscle weakness**, resulting from acquired myopathy, may be seen late in the illness.
- **Sleep apnea** is common.
- **Organomegaly,** especially **thyromegaly**, is common, but hyper thyroidism is very rare unless the **tumor co-secretes TSH.**

Acromegaly

- GH excess is also a hyper metabolic condition and, as a result, acromegalic patients may complain of **excessive sweating** or **heat intolerance**.
- The **features of acromegaly** slowly develop over time, and the patient (or their family) may be unaware that changes in **physiognomy** have occurred.
- **Local effects** of the tumor (**headache or visual complaints**) or symptoms related to the loss of other anterior pituitary hormones (**hypopituitarism**).
- A careful, retrospective review of older photographs may be crucial in differentiating coarse features.

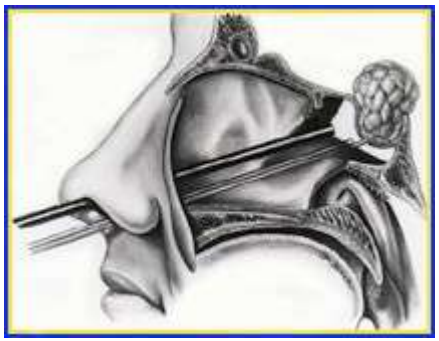
Acromegaly

- **If left untreated, increased risk of heart disease, resulting from the combination of HT, CAD , and D /IR . Risk of developing cancer** (regular colonoscopy) are recommended.

- **Co secretion of prolactin** can be seen in up to 40% of patients with acromegaly. Only a **few TSH/GH–secreting tumors** have also been reported.

Treatment of acromegaly

- The goal of treatment is tumor ablation, with continued function of the remainder of the pituitary.
- Transphenoidal adenomectomy is the procedure of choice.



Treatment of acromegaly

- **GH-producing tumors** may be **too large** or may **invade into local** structures that prevent complete surgical extirpation, and the patient is left with a smaller, but hormonally active tumor.
- **External beam or focused irradiation** is frequently used at this point, but it may take several years before GH levels decline.

Treatment of acromegaly

- **Somatostatin analogs and dopaminergic agonists**, may be employed for GH suppression, but it is important to note that dopaminergic agonists are only effective if the tumor **co secretes prolatin**.

- **Pegvisomant**, a **GH receptor antagonist** approved as an adjuvant in the medical management of acromegaly.

Growth Hormone Suppression Test, and Growth Hormone Stimulation Test

- Adult: *Males:* <5 ng/mL

  Females: <10 ng/mL
- Children: 0–10 ng/mL
- Newborns: 10–40 ng/mL
- Red-top tube.

■ Growth hormone is produced in episodic bursts by the pituitary gland.

■ Assessed to evaluate possible dwarfism, growth retardation, or gigantism in children and acromegaly in adults.

Growth Hormone Deficiency

- GH deficiency occurs in both children and adults.
- **In children**, it may be **familial** or it may be due to **tumors**, such as **craniopharyngiomas**.
- **In adults**, it is a result of **structural or functional** abnormalities of the pituitary; however, a **decline in GH** production is **an predictable consequence of aging.**
- Although GH deficiency in children is manifest by growth failure, **not all patients** with **short stature** have GH deficiency.

Growth Hormone Deficiency

- There have been **several genetic defects** identified in the GH axis.

1- The more common type is a recessive mutation in the **GHRH gene** that causes a failure of GH secretion.

2- A **rarer mutation**, loss of the GH gene itself.

3- Mutations that cause **GH insensitivity**.

These mutations may involve

- *GH receptor,
- *IGF-I biosynthesis,
- *IGF-I receptors,
- * defects in GH signal transduction.

Adult GH deficiency syndrome

- Patients who have **complete or even partial failure** of the anterior pituitary.
- **The symptoms** include social withdrawal, fatigue, loss of motivation, and a diminished feeling of well-being.
- **Osteoporosis** and **alterations in body composition** (reduced lean body mass) are frequent concomitants of adult GH deficiency.
- **GH replacement therapy** (recombinant human GH)

PROLACTIN

- ***Prolactin*** is **structurally related to GH and human placental lactogen.**
- Considered a **stress hormone**, it has vital functions in relationship to reproduction.
- Prolactin is classified as a **direct effector hormone** because it **has diffuse target tissue** and **lacks a single endocrine end organ**.
- **Prolactin** is **unique** among the anterior pituitary hormones because its major mode of hypothalamic regulation is **tonic inhibition** rather than intermittent stimulation.

- **Prolactin inhibitory factor (PIF)** considered a **polypeptide hormone** capable of inhibiting prolactin secretion; **dopamine**, however, is the only neuroendocrine signal that inhibits prolactin.

- Any compound that **affects dopaminergic activity** in the median eminence of the hypothalamus will also alter prolactin secretion.

Medications cause hyperprolactinemia

- phenothiazines, butyrophenones, metoclopramide, reserpine, tricyclic antidepressants, α-methyldopa, and antipsychotics that antagonize the dopamine D2 receptor.

Causes of hyperprolactinemia

- Any **disruption of the pituitary stalk** (tumors, trauma, inflammation) causes an elevation in prolactin as a result of interruption of the **flow of dopamine** from the hypothalamus to the lactotropes, the pituitary prolactin- secreting cells.
- **TRH** directly stimulates prolactin secretion, and increases in TRH (in primary hypothyroidism) elevate prolactin levels.
- **Estrogens** also directly stimulate lactotropes to synthesize prolactin.
- **Pathologic (hyperprolactinemia)** associated with chest wall injuries, renal failure & PCOS.

Causes of hyperprolactinemia

- **Physiologic stressors** (exercise and seizures) also elevate **prolactin**.
- primary regulation of prolactin secretions is **tonic inhibition** (dopamine),also **regulated by GRH, TRH& vasoactive intestinal polypeptide**.
- **Stimulation of breasts**, as in nursing, causes the release of **prolactin secreting hormones** from the hypothalamus through a spinal reflex activate the physiologic effect of prolactin **is lactation**.

Causes of hyperprolactinemia

- The usual consequence of **prolactin excess** is hypogonadism, either by suppression of **gonadotropin secretion** from the pituitary or by inhibition of **gonadotropin action** at the gonad.

- The **suppression of ovulation** seen in lactating postpartum mothers.

Prolactinoma

The most common type of functional pituitary tumor.

The clinical presentation depends on the **age and gender** and the **size of the tumor**.

Premenopausal women most frequently complain of menstrual irregularity / amenorrhea, infertility, or galactorrhea.

Men or postmenopausal women generally present with symptoms of a pituitary mass, such as headaches or visual complaints.

Occasionally, a **man** may present with reduced libido or complaints of erectile dysfunction.

The reason(s) for the varied presentations of a prolactinoma are **somewhat unclear** but likely relate to **alteration in menses** or the sudden onset of a **breast discharge in younger women**.

Other Causes of Hyperprolactinemia

- **Substantial elevations** in prolactin (>150 ng/mL) degree of elevation in prolactin is correlated with **tumor size**.
- **Modest elevations** in prolactin (25–100 ng/mL): pituitary stalk interruption, use of dopaminergic antagonist medications, primary thyroidal failure, renal failure, PCOS.
- **Significant hyperprolactinemia** is also encountered during **pregnancy**.

Other Causes of Hyperprolactinemia

- Prolactin is a **23-kD peptide**; however, **a 150-kD** form may also be secreted.
- This larger prolactin molecule has a markedly **reduced biologic potency**.
- If the 150-kD form of prolactin predominates, this is called **macro-prolactinemia.**
-

Clinical evaluation of hyperprolactinemia

- 1- If a **pituitary tumor** is suspected, a careful assessment of anterior pituitary function (basal cortisol, LH, FSH, estradiol or testosterone).

- 2- Evaluation of **sellar anatomy** with a high-resolution **MRI** should be obtained.

Management of Prolactinoma

- The different therapeutic options include

1-simple observation,

2- surgery,

3- radiotherapy,

4- medical management with dopamine agonists.

However, the **management of prolactinoma** also depends on the size of the tumor (**macroadenomas** [tumor size >10 mm] are less likely to be "cured" than are **microadenomas** [tumor size <10 mm]) and the preferences of the patient.

Management of Prolactinoma

- **Dopamine agonists** are the most commonly used therapy for **microprolactinomas**.
- **Tumor shrinkage** is noted in more than 90% of patients treated with bromocriptine mesylate or cabergoline.
- **Both drugs** also shrink prolactin-secreting **macroadenomas.**
- A **continuation of menses** and **restoration of fertility** is also frequently seen **during medical therapy**.

Management of Prolactinoma

- **Neurosurgery** is not a primary mode of prolactinoma management.
- **Neurosurgery used for:**

  -pituitary tumor apoplexy (hemorrhage),

  - acute visual loss due to macroadenoma,

  - cystic prolactinoma,

  - or intolerance to medical therapy.

- **External beam radiotherapy** is generally reserved for high surgical risk patients with locally aggressive macroadenomas who are unable to tolerate dopamine agonists.

Idiopathic Galactorrhea

- Lactation occurring in women with normal prolactin levels is defined as ***idiopathic galactorrhea***.
- This condition is usually seen in women who have been **pregnant several times** and has no pathologic implication.

HYPOPITUITARISM

- The failure of either the **pituitary or hypothalamus** results in the loss of anterior pituitary function.
- Complete loss of function is termed ***pahypopituitarism***; loss of only a single pituitary hormone, ***monotropic hormone deficiency***.
- The **loss of a tropic hormone** (**ACTH, TSH, LH, and FSH**) is reflected in function cessation of the affected endocrine gland.
- **Loss of the direct effectors** (**GH and prolactin**) may not be readily apparent.

laboratory diagnosis of hypopituitarism

- The **primary failure of an endocrine gland** that is accompanied by **dramatic increases** in circulating levels of the corresponding **pituitary tropic hormone**, **secondary failure (hypopituitarism)** is associated with low or normal levels of **tropic hormone**.
- **In primary hypothyroidism**, the circulating levels of thyroxine are low and TSH levels may exceed 200 µU/mL (normal, 0.4–5.0).
- As a result of **pituitary failure in hypothyroidism**, TSH levels are inappropriately low and typically less than 1.0 µU/mL.

Causes of hypopituitirsm

1. Pituitary tumors
2. Parapituitary/hypothalmic tumors
3. Trauma
4. Radiation therapy/surgery
5. Infarction
6. Infection
7. Infiltrative disease
8. Immunologic
9. Familial
10. Idiopathic

Treatment of Panhypopituitarism

- Replacement therapy for panhypopituitarism is the same as for **primary target organ failure**.

  (thyroxin, glucocorticoid, and sex steroids)

- **Replacement** becomes more complicated in panhypo-pituitary patients who desire **fertility**.
- **Pulsatile GnRH infusions** have **induced puberty** and **restored fertility** in **Kallmann's syndrome.**
- **Gonadotropin preparations** have **restored ovulation** /s**permatogenesis** in people with **gonadotropin deficiency.**

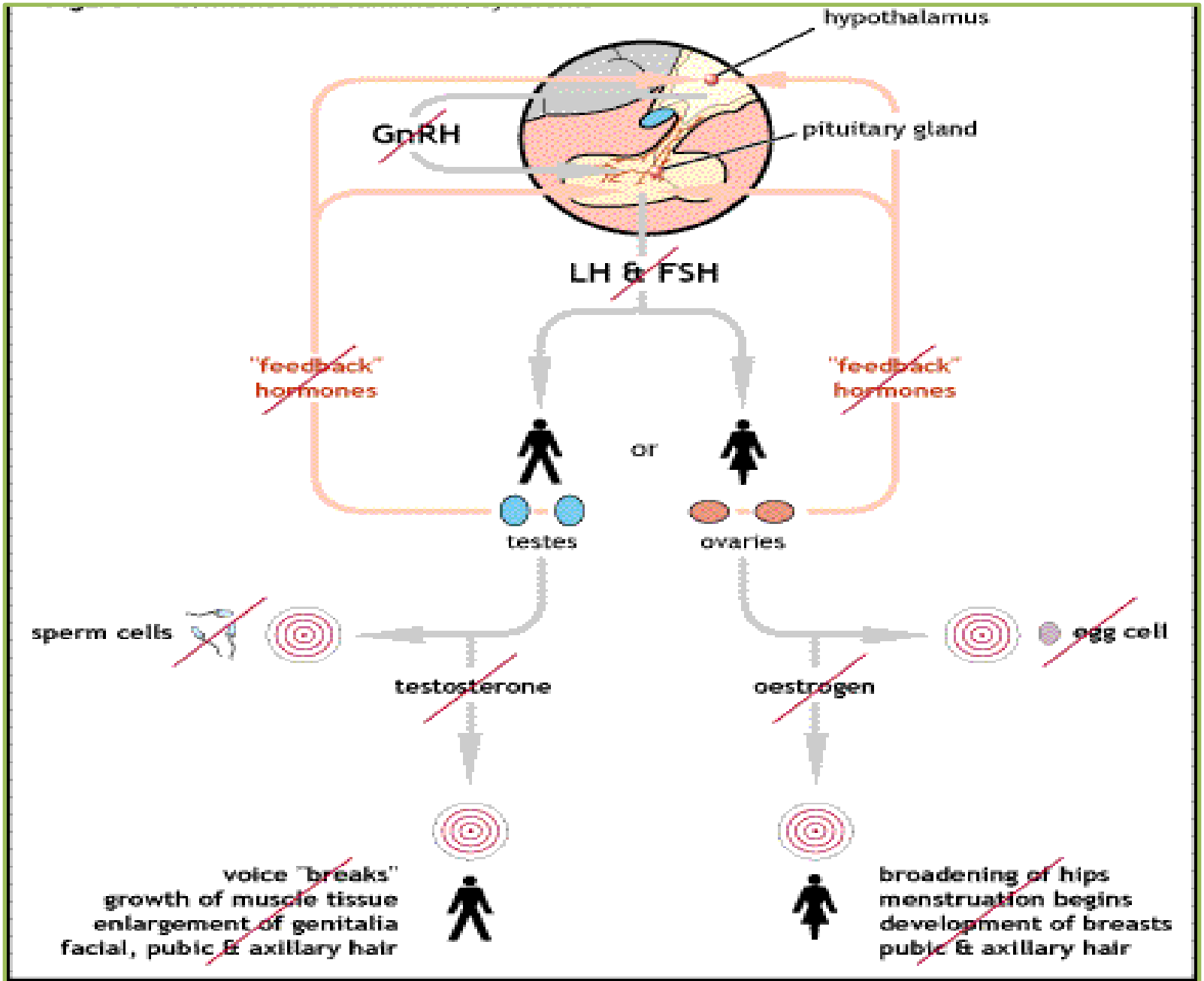
Kallmann syndrome

- Is a rare **genetic condition** that is characterized by a **failure to start or to complete puberty**
- It is also accompanied by a **lack of sense of smell** (anosmia) or a highly **reduced sense of smell** (hyposmia).
- The condition can occur in **both males and females** but is more commonly diagnosed in males.
- **Left untreated**, patients with Kallmann syndrome will almost invariably be infertile.
- **Kallmann syndrome** occurs due to a failure of the **hypothalamus to release GnRH** .
- Kallmann syndrome is a form of **hypogonadotropic hypogonadism (HH).**

Kallman syndrome

- failure of one kidney to develop (unilateral renal agenesis), abnormalities of bones in the fingers or toes, a cleft lip with or without an opening in the roof of the mouth (a cleft palate), abnormal eye movements, hearing loss, and abnormalities of tooth development.

Hormones in Kallman



POSTERIOR PITUITARY HORMONES

- **Vasopressin** and oxytocin, small peptide hormones are synthesized in the supra optic and hormones are synthesized outside of the hypothalamus in various tissue, and it is plausible they have an autocrine or a paracrine function.

Oxytocin

- Oxytocin is a **cyclic nonapeptide**, with a disulfide bridge connecting amino acid residues **1 and 6.** As a posttranslational modification, the C-terminus is amidated.
- Oxytocin has a **critical role in lactation** and likely plays a **major role in labor and parturition**.
- (Pitocin) is used in obstetrics to induce labor.
- Oxytocin have effects on pituitary, renal, cardiac, and immune function.

Vasopressin

- is a cyclic nonapeptide with an identical disulfide bridge; it differs from oxytocin by only two amino acids.
- Vasopressin's major action is to **regulate renal free water excretion** and, therefore, has a **central role in water balance**.
- ***The vasopressin receptors*** in the **kidney (V2)** in the renal collecting tubules and the ascending limb of the loop of Henle.
- They are coupled to **adenylate cyclase if** activated lead to induce insertion of **aquaporin-2 (a water channel protein)** into the tubular luminal membrane.
- ***Vasopressin*** is also a **potent pressor agent** and effects blood clotting by **promoting factor VII release from hepatocytes and Von Willebrand factor** release from the endothelium.
- Vasopressin receptors **(V1a and V1b)** are coupled to **phospholipase C.**

Vasopressin

- **Hypothalamic osmoreceptors and vascular baroreceptors** regulate the release of **vasopressin from the posterior pituitary**.
- **The osmoreceptors** are extremely sensitive to even small changes in plasma osmolality, with an average osmotic threshold for vasopressin release in humans of 284 mOsm/kg.
- As **plasma osmolality increases**, **vasopressin** secretion increases. The consequence is a **reduction in renal free water clearance**, a lowering of plasma osmolality a **return to homeostasis**.

Vasopressin

- The **vascular baroreceptors** (located in the left atrium, aortic arch, carotid arteries) initiate vasopressin release in response to a **fall in blood volume** or **blood pressure**.
- A **5-10% fall in arterial blood pressure** in normal humans will trigger vasopressin release; however, in opposite to an osmotic stimulus, the vasopressin response to a **baroreceptor-induced stimulus** is exponential.

Diabetes insipidus (DI)

- Characterized by copious production of urine (polyuria) and intense thirst (polydipsia), is a consequence of **vasopressin deficiency.**
- However**, total vasopressin deficiency** is unusual, and the typical patient presents with a **partial deficiency.**
- **Causes of hypothalamic DI:** autoimmunity to vasopressin-secreting neurons, trauma, diseases affecting pituitary stalk function, and various CNS or pituitary tumors.
- A sizable percentage of patients (up to 30%) will have **idiopathic DI**.

Diagnosis of DI

- **Water deprivation test** in which fluids are withheld from the patient and serial determinations of **serum and urine osmolality** are performed in an attempt to document the patient's ability to conserve water.

- **Vasopressin or a synthetic analog (Desmopressin dDAVP)** and assess the patient's response; amelioration of both polyuria and polydipsia would be considered a positive response, and a presumptive diagnosis of DI is made.

- **Primary polydipsia** (compulsive water drinking), a profound hypoosmolar state **(water intoxication)** can result due to the continued ingestion of copious amounts of fluids and a reduced renal excretion of free water.

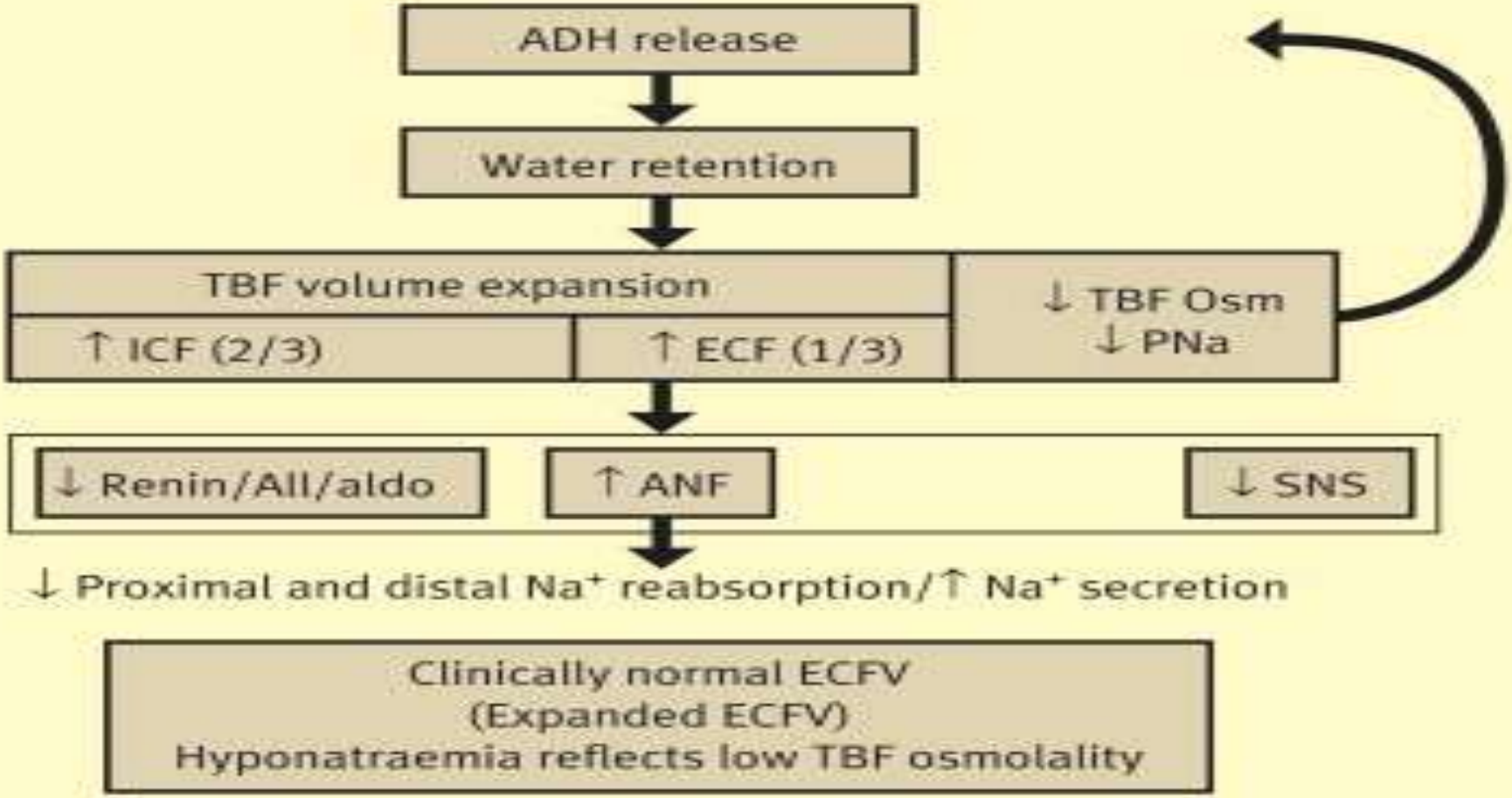
- **Conivaptan**, a vasopressin V2 receptor antagonist, has been approved for the management of euvolemic hyponatremia due to vasopressin excess.

Condition	Urine osmolality in m Osm/kg, after H2O deprivation[	After desmopressin or vasopressin
Normal	> 800	> 800 (<10% increase)
a defect in ADH production (central/neurogenic DI)	< 300	> 800 (>50% increase)
a defect in the kidneys' response to ADH production nephrogenic DI	< 300	< 500 (<50% increase)
excessive intake of H2O primary polydipsia	> 500	> 500 (<10% increase)

Syndrome of inappropriate antidiuretic hormone secretion (SIADH)

- is characterized by excessive release of ADH from the posterior pituitary gland or another source.
- The **increase in blood volume** (hypervolemia) often results in **dilutional hyponatremia** in which the plasma sodium levels are lowered and total body fluid is increased.
- Although the sodium level is low, SIADH is brought about by an excess of water rather than a deficit of sodium.

The Syndrome of Inappropriate Antidiuretic Hormone Secretion (SIADH)



ADH, antidiuretic hormone; TBF, total body fluid; ICF, intracellular fluid; ECF, extracellular fluid; aldo, aldosterone; ANF, atrial natriuretic factor; SNS, sympathetic nervous system; ECFV, extracellular fluid volume.

ADH Suppression Test

ADH: 1–5 pg/mL; SI units: <1.5 ng/L
ADH suppression test: 80% of water load excreted in 5 hr;
Urine osmolality ≥100 mmol/kg;
Urine to serum osmolality ratio >100;
Urine specific gravity <1.003.

Red-top *plastic* tube

- ADH controls the amount of water reabsorbed by the kidney.
- Inadequate ADH secretion results in (DI).
- Excess secretion of ADH related to various cancers (lung, pancreas, urinary tract, blood) results in syndrome of inappropriate ADH (SIADH).

Diabetes Insipidus		SIADH
- High Urinary Output		- Low Urinary Output
- Low Levels of ADH		- High Levels of ADH
- Hypernatremia	VS	- Hyponatremia
- Dehydrated		- Over Hydrated
- Lose too much fluid		- Retain too much fluid

* Both will present with excessive thirst

The Reproductive System

Lecture clinical chemistry
2018-2019

The reproductive system

- The reproductive system is responsible not only for the production of hormones, but also for maturation of the gamets in the gonads.
- The hypothalamus secretes (GnRH), which regulates the secretion of the pituitary **(LH), (FSH)& dopamine**, a neurotransmitter, which also controls prolactin secretion.

Anterior pituitary hormones

- **(LH and FSH)** secreted by pituitary basophil cells, control the function and secretion of hormones by the testes and ovaries.
- The secretion of **GnRH is pulsatile** in turn, is that of **LH and FSH.**
- Although there is only one releasing hormone, secretion of LH and FSH does not always occur in parallel and may be modified by feedback from the circulating concentrations of **gonadal androgens or estrogens**.
- **LH** primarily stimulates the production of hormones by the gonads, **FSH** stimulates the development of the germ cells

Anterior pituitary hormones

Prolactin, secreted by acidophil cells, is important during pregnancy and the post-partum period.

1-It stimulates **breast epithelial cell** proliferation and induces **milk production**.

2-Prolactin differs from all other pituitary hormones in its **method of control.**

3-Secretion is **inhibited, not stimulated,** by dopamine (**prolactin inhibitory factor**; PIF); therefore, impairment of hypothalamic control causes **hyperprolactinaemia**. *Its secretion is regulated by a short feedback loop between pituitary prolactin and hypothalamic dopamine*.

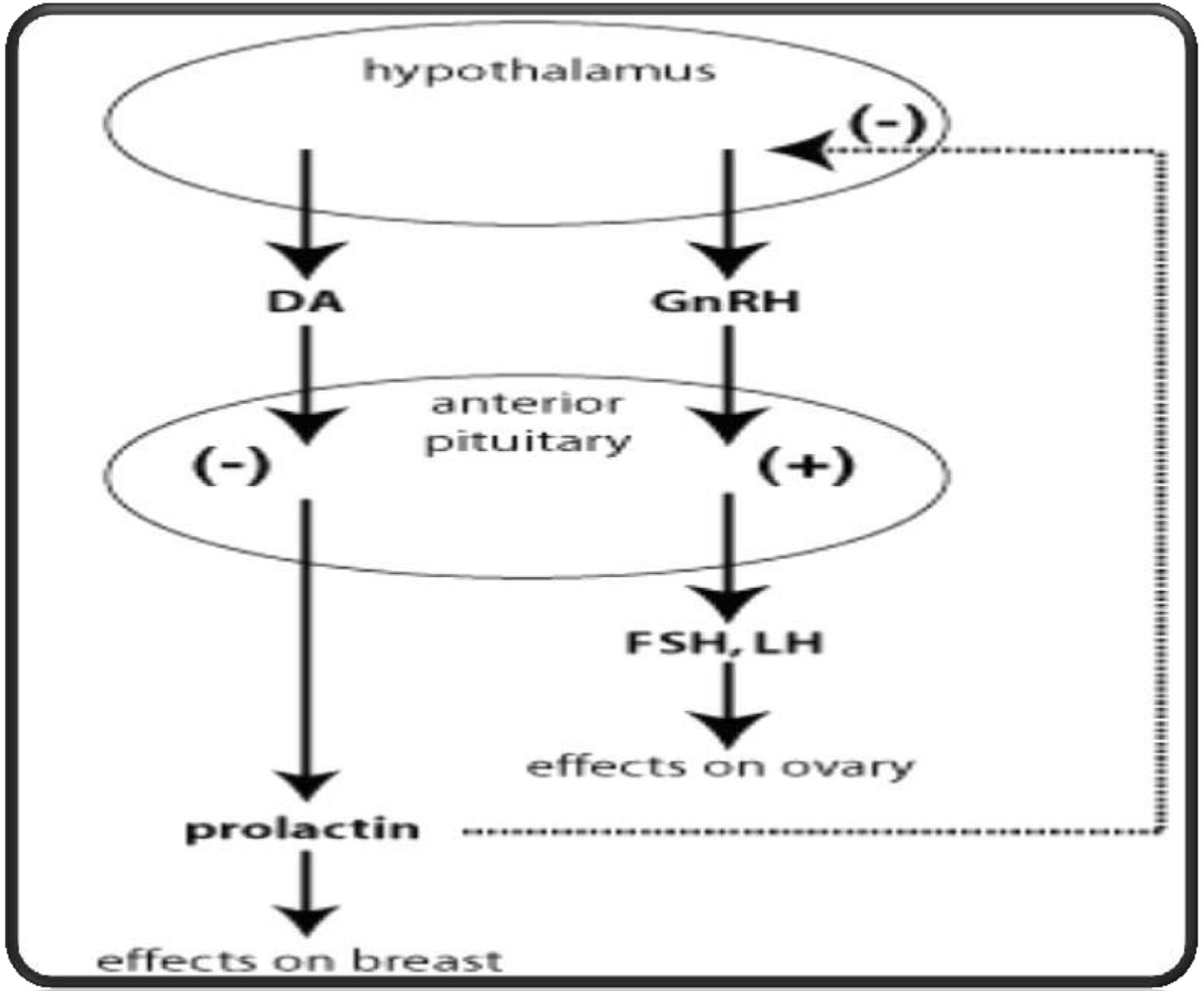
Anterior pituitary hormones

4-Estrogens stimulate the proliferation of pituitary lactotroph cells.

- Circulating prolactin concentrations are normally higher in pregnancy and increase during suckling as a result of the **action of vasoactive intestinal peptide (VIP)** and **also high estrogen concentrations in pregnancy.**

- **(TRH)& (TSH)** stimulates the secretion of prolactin, this action be important in pathological conditions only.

- Similar factors affect prolactin and GH secretion.
- Secretion of both is **pulsatile and increases during sleep** and in response to physical and psychological stress.


hypothalamus
(-)
DA
GnRH
anterior
pituitary
(-)
(+)
FSH, LH
effects on ovary
prolactin
effects on breast

HYPERPROLACTINAEMIA

- This is an important cause of amenorrhoea, osteoporosis, infertility and possibly breast cancer.

- **High plasma prolactin** concentrations inhibit the normal pulsatile release of **GnRH** and inhibit **gonadal steroid hormone** synthesis.
- About a third of patients with hyperprolactinaemia have galactorrhoea.

- The plasma prolactin concentration is raised during pregnancy and lactation. Plasma prolactin concentrations are higher in females than in males and decrease **post menopause.**

- **Samples for prolactin estimations** should be taken at least 2–3 h after waking in order to eliminate the misleading elevated plasma concentrations found during sleep; the stress of vein puncture may also cause prolactin secretion.

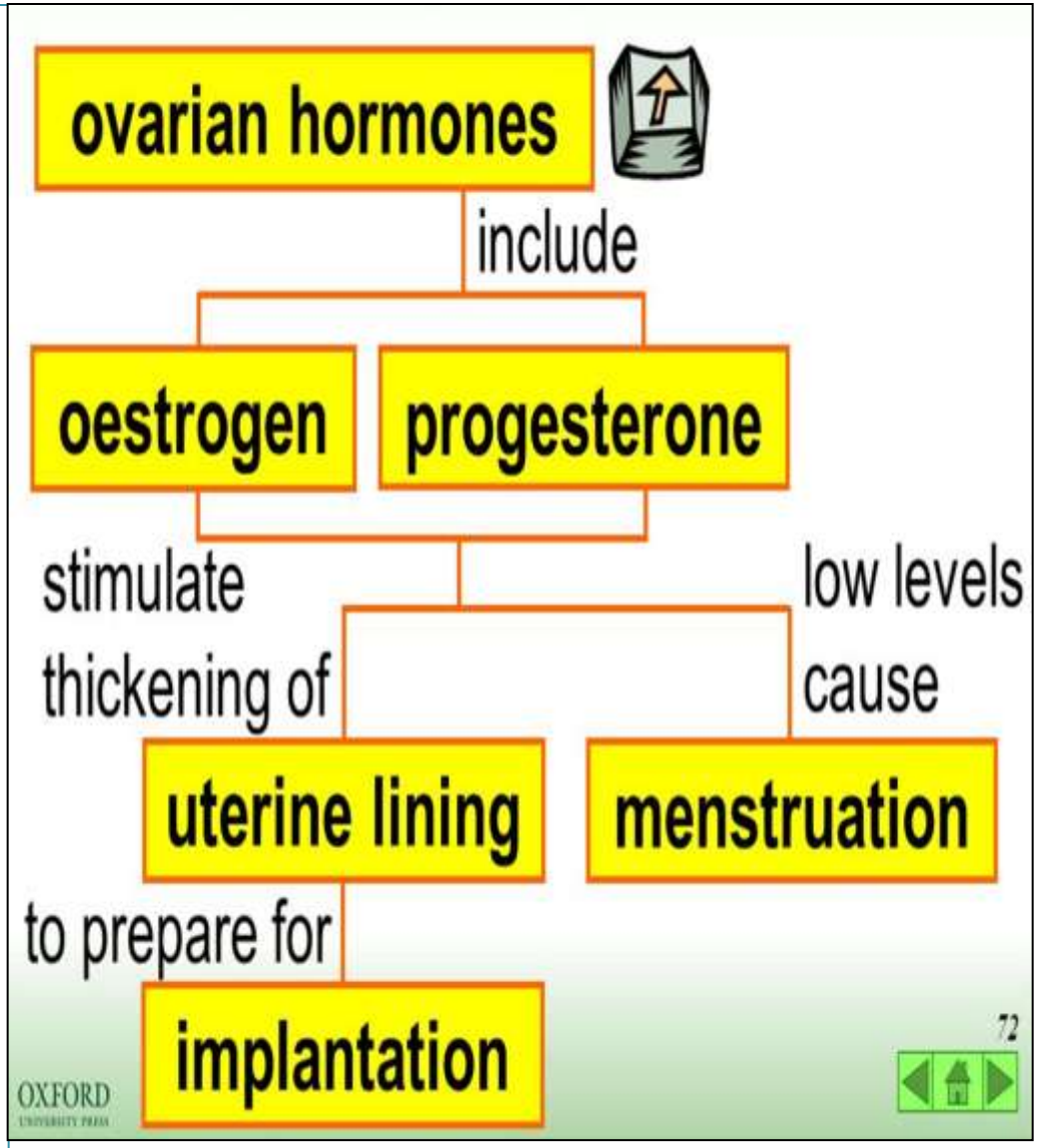
Some causes of hyperprolactinaemia

- ☐ **Physiological,:** stress, pregnancy or lactation
- ☐ Failure of **hypothalamic inhibitory factors** to reach the anterior pituitary gland due to: Damage to the pituitary stalk by non-prolactin secreting tumors of the pituitary gland or hypothalamus; Surgical section of the pituitary stalk
- ☐ **Other pituitary tumors**: Microadenomas or Macroadenomas
- ☐ **Drugs:** Estrogens; Dopaminergic antagonists, e.g. Phenothiazines, Haloperidol, Metoclopramide, Methyldopa, Reserpine
- ☐ **PCOS**
- ☐ **Chronic kidney disease** (due to reduced plasma prolactin clearance)
- ☐ **Severe primary hypothyroidism** (due to anterior pituitary stimulation by high TRH concentrations).
- ☐ **Macroprolactinaemia**

- Pituitary imaging with **CT or MRI** may show a tumor.
- Dopamine receptor agonists such as **bromocriptine or cabergoline** are used to lower prolactin concentrations.
- Sometimes pituitary **surgery** is needed to remove a pituitary tumor.

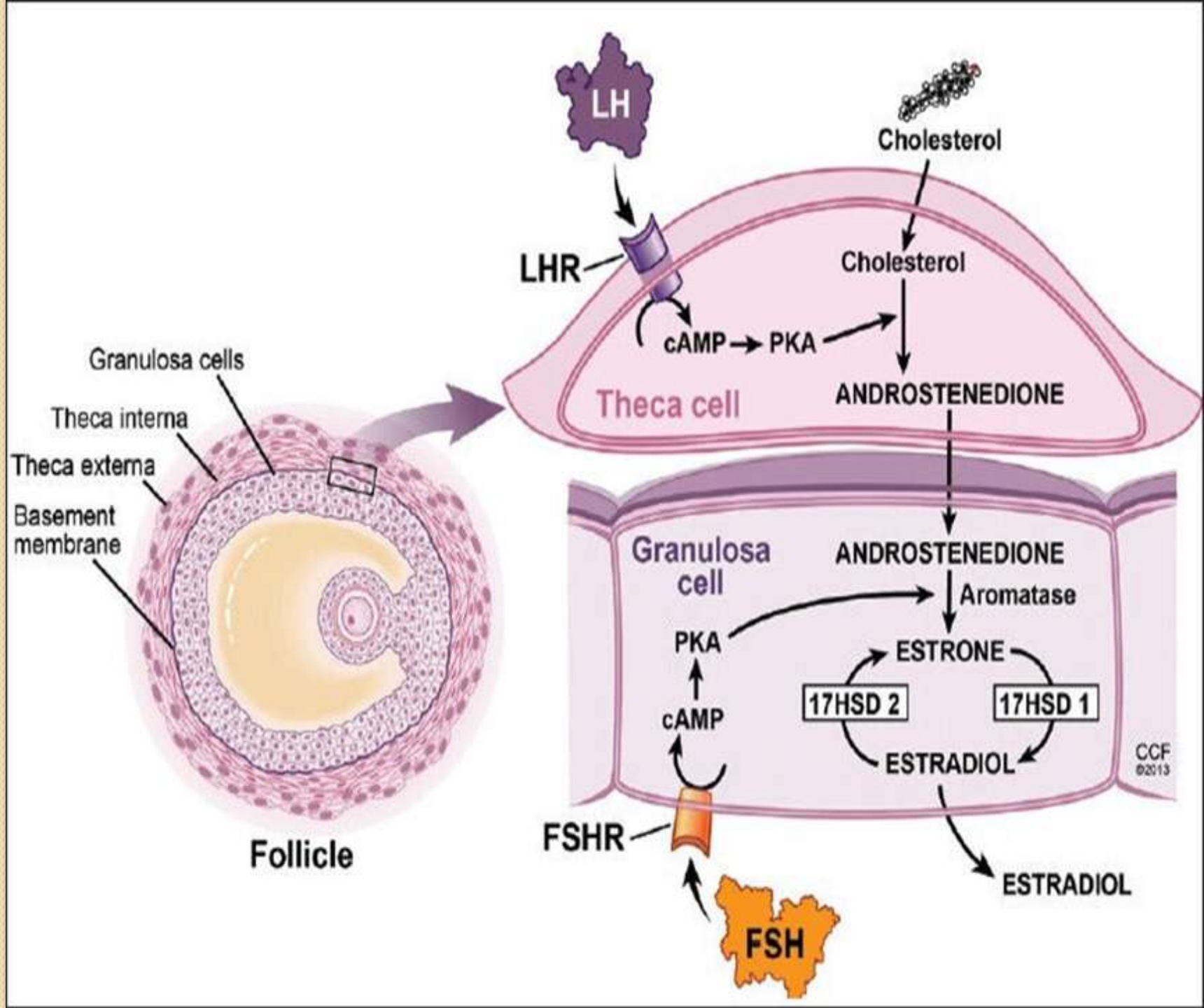
Ovarian hormones

- Estrogens, progesterone and androgens are secreted by the ovarian follicles of the ovaries, which consist of germ cells (ova) surrounded by granulosa and theca cells.



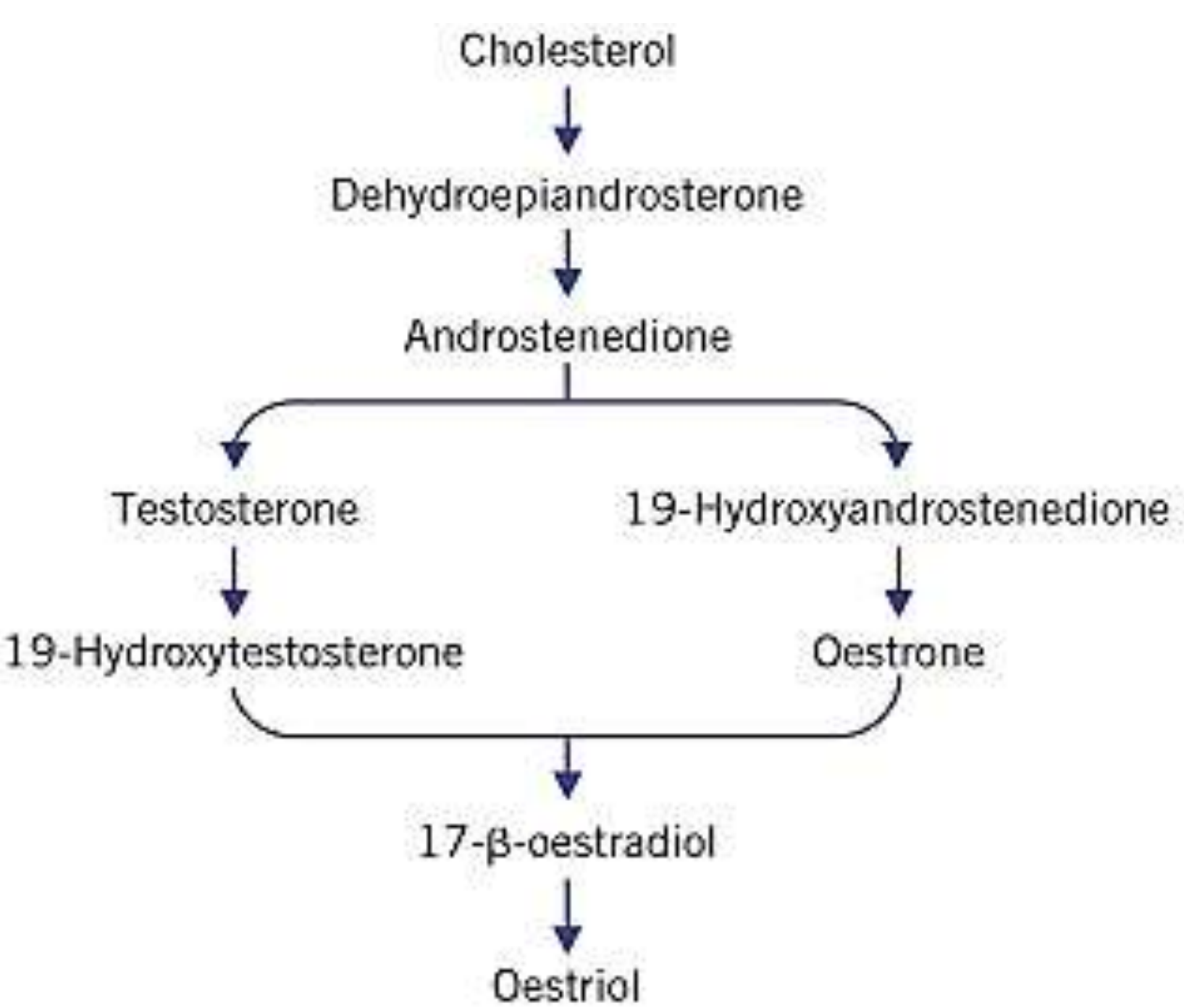
Ovarian hormones

- **Androgens** (C19 steroids), synthesized by theca cells, are converted into estrogens (C18 steroids) in the granulosa cells, a process that involves aromatization of the A ring and the loss of the C-19 methyl group.
- **Estradiol** is the most important ovarian estrogen.
- The **liver and subcutaneous fat** convert ovarian and adrenal androgens to estrone (peripheral aromatization).
- Both **estradiol and estrone** are metabolized to the relatively **inactive estriol.**


LH
Cholesterol
LHR
Cholesterol
cAMP → PKA
Granulosa cells
Theca interna
Theca externa
Basement membrane
Theca cell
ANDROSTENEDIONE
Granulosa cell
ANDROSTENEDIONE
Aromatase
PKA
ESTRONE
17HSD 2
17HSD 1
cAMP
ESTRADIOL
CCF ©2013
FSHR
ESTRADIOL
FSH
Follicle

Ovarian hormones

- **Estrogens** are essential for the development of female secondary sex characteristics and for normal menstruation, and their concentration in plasma in children is **very low.**
- **Androstenedione** is the main androgen secreted by the ovaries. It can be converted to **estrone**, or to the more **active testosterone** and then to **estrdiol.**
- A small amount of **testosterone** is secreted directly by the ovaries. Plasma concentrations in women are about a **tenth of those in men**.


Cholesterol
Dehydroepiandrosterone
Androstenedione
Testosterone
19-Hydroxyandrostenedione
19-Hydroxytestosterone
Oestrone
17-β-oestradiol
Oestriol

Ovarian hormones

- **Progesterone** is secreted by the **corpus luteum** during the luteal phase of the menstrual cycle and by the **placenta.**
- It **prepares the endometrium of the uterus** to receive a fertilized ovum and is necessary for the maintenance of early pregnancy.
- It also is **pyrogenic** and increases the basal body temperature.
- In plasma, only about **2% of progesterone** is unbound or free, the majority being bound to albumin and transcortin.
- **Inhibin** from granulosa cells inhibits FSH secretion while **activin** enhances the action of FSH and LH.

Ovarian hormones

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Testicular hormones

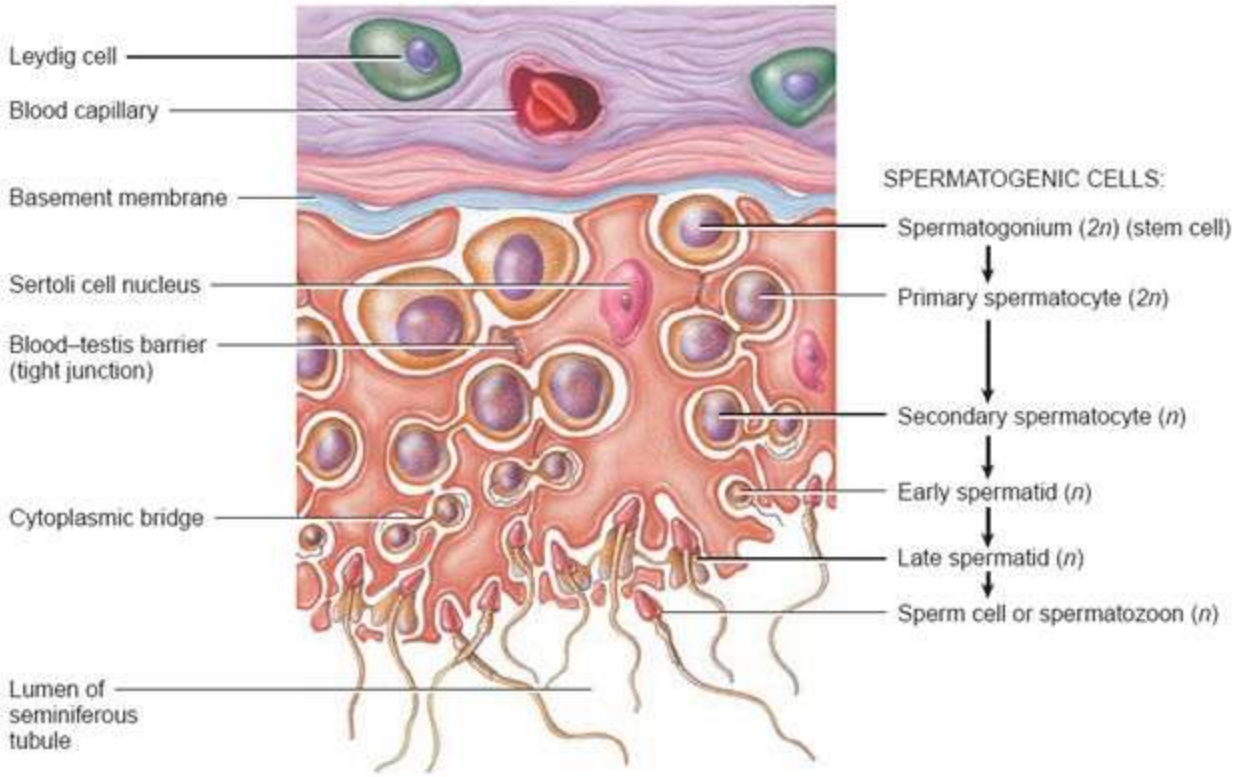
- **Testosterone** is secreted by the Leydig cells, which lie in the interstitial tissue of the testes between the seminiferous tubules.
- The production of **testosterone** is stimulated by LH and it, in turn, inhibits LH secretion by negative feedback.
- **Inhibin** is a hormone produced by the Sertoli cells, part of the basement membrane of the seminiferous tubules, during germ cell differentiation and spermatogenesis.

These processes require testosterone and are stimulated by FSH.

- **Inhibin** controls FSH secretion by negative feedback and **activin** enhances spermatogenesis.

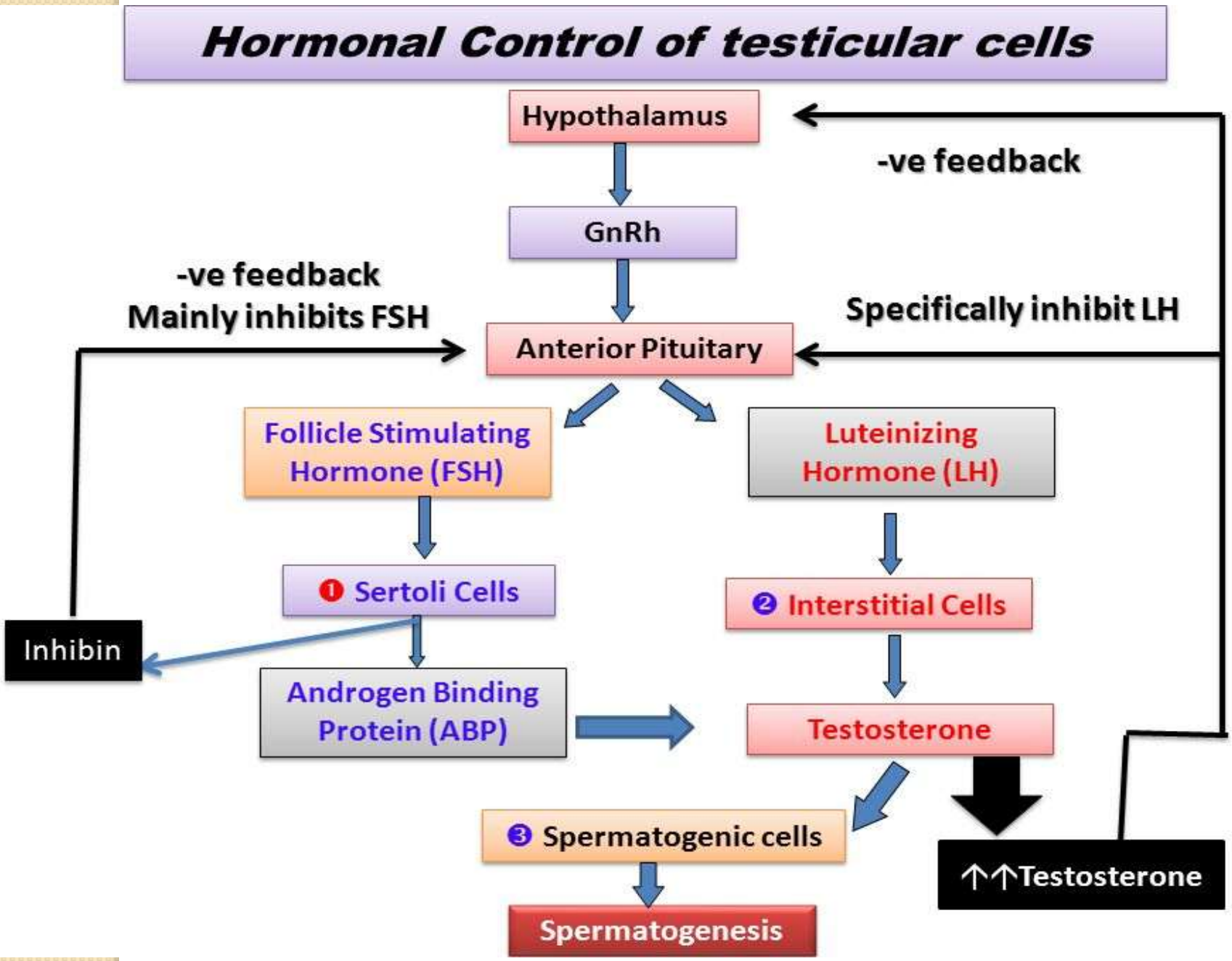
Endocrine Cells in Testes

- **Leydig Cell**
  - Testosterone
- **Sertoli Cell**
  - Inhibin （抑制素）
  - Activin （激活素）



Testicular hormones

- Testosterone is involved in sexual differentiation, the development of secondary sexual characteristics, **spermatogenesis and anabolism**.
- In the male, the effects of testosterone depend on intracellular conversion to the even more potent androgen **dihydrotestosterone** by the 5 α reductase in target cells.


Hormonal Control of testicular cells
Hypothalamus
-ve feedback
GnRh
-ve feedback
Mainly inhibits FSH
Specifically inhibit LH
Anterior Pituitary
Follicle Stimulating Hormone (FSH)
Luteinizing Hormone (LH)
❶ Sertoli Cells
❷ Interstitial Cells
Inhibin
Androgen Binding Protein (ABP)
Testosterone
❸ Spermatogenic cells
↑↑Testosterone
Spermatogenesis

SEXUAL DEVELOPMENT FROM CONCEPTION IN FEMALES AND MALES

- Chromosomal sex is determined at fertilization by the chromosomes present in the ovum and sperm, each of which contributes 22 autosomes and one sex chromosome, X or Y.
- Normal males have a 46,XY karyotype, and normal females 46,XX.
- Abnormalities occurring at this stage may **result in defective gonadal development,** as occurs in **Klinefelter's syndrome** in males (47,XXY) or **Turner's syndrome** in females (45,X0).
- The sex chromosomes determine whether the **primitive gonads** become testes or ovaries. In addition to the chromosomal sex determination, environmental factors (**hormonal levels**) also participate.

The female

- Development of female characteristics
- In the absence of a Y chromosome, the fetus starts to develop female characteristics at **about 12 weeks of gestation**.
- If **androgens** are produced at this stage in **(CAH),** masculinization of the external genitalia may occur, causing female **pseudo hermaphroditism.**
- Proliferation of fetal germ cells produces several million oocytes.
- By late fetal life, all the germ cells have degenerated and no more oocytes can be produced.
- Those oocytes that are present enter the first stage of meiosis and their numbers decline throughout the rest of the intrauterine period and childhood; the inability to replenish them explains the limit to the span of reproductive life in women, in contrast to the continuous ability of men to produce sperm.
- An abnormally high rate of decline leads to premature menopause.

Female puberty

- At the onset of puberty, **gonadotrophin** secretion increases, as it does in the male. **Ovarian estrogen** secretion rises and stimulates the development of female secondary sex characteristics and the onset of menstruation (menarche).

Normal gonadal function

- At puberty the ovaries contain between 100 000 & 200 000 primordial follicles. During each menstrual cycle a small number develop, but usually only one reaches maturation, which is extruded from the ovary as an ovum (ovulation), and is ready for fertilization.
- The menstrual cycle is regulated by changing hormone concentrations and by changing sensitivity of ovarian tissue.

Normal gonadal function

- Follicular (pre-ovulatory) phase

- At the beginning of the menstrual cycle, ovarian follicles are undeveloped and plasma estradiol concentrations are low. The secretion of LH and FSH increases because of diminished negative feedback by estrogens.
- Together, LH and FSH cause the growth of a group of follicles. By about the seventh day of the cycle, one follicle becomes especially sensitive to FSH and matures, while the rest atrophy. LH also stimulates estradiol secretion, the plasma concentrations of which rise steadily. This stimulates the regeneration of the endometrium.

Normal gonadal function

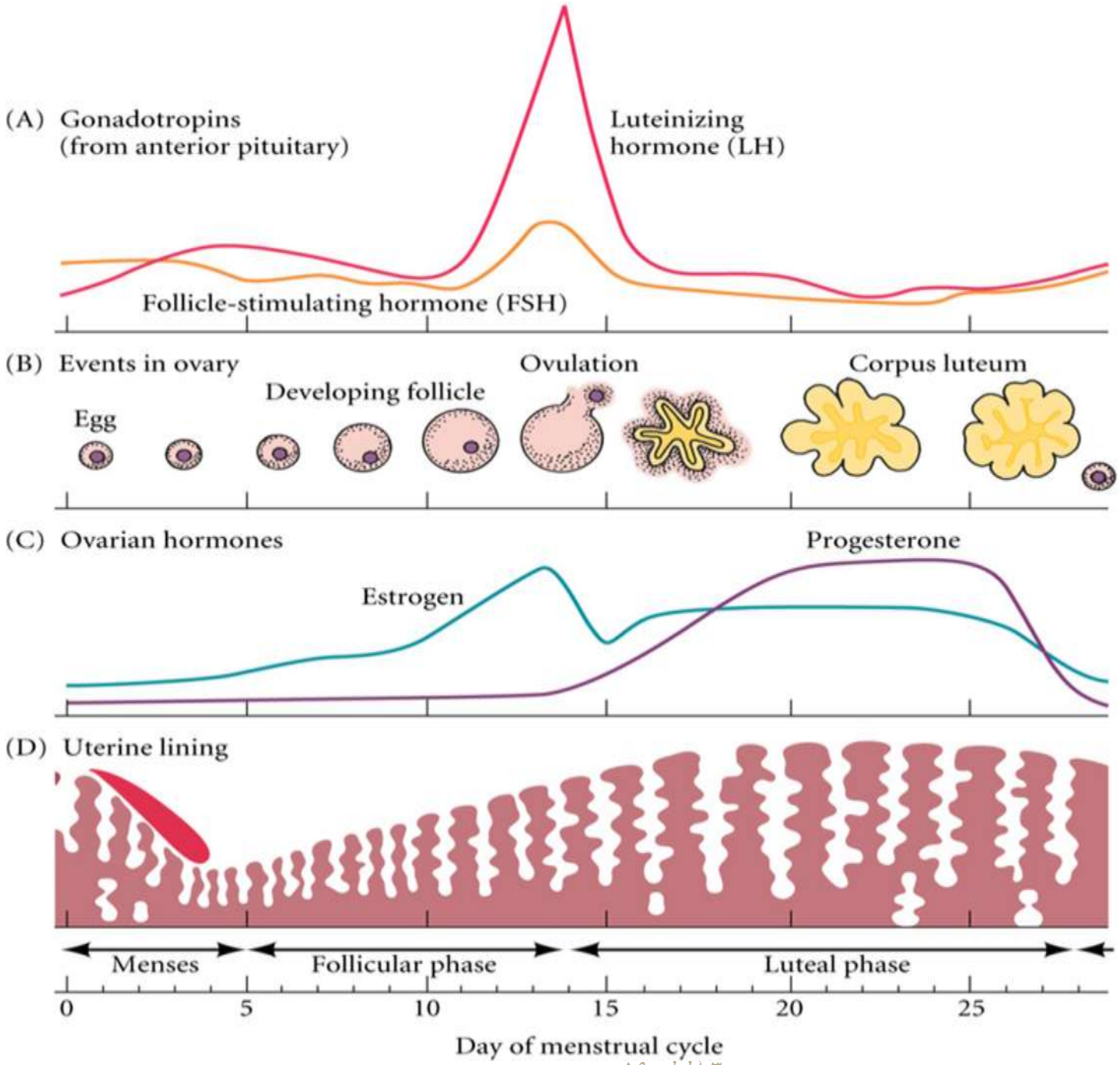
- Ovulatory phase

- The rapid development of the dominant follicle and the rise in plasma estradiol concentration trigger a surge of LH release from the anterior pituitary gland by positive feedback.
- Ovulation occurs about 16 h later.
- Combined oral contraceptives suppress gonadotrophin plasma concentrations.

Normal gonadal function

- Luteal (post-ovulatory or secretory) phase

- After ovulation, the high LH concentration stimulates the granulosa cells of the ruptured follicle to luteinize and form the corpus luteum, which synthesizes and secretes **progesterone and estradiol.**
- Progesterone is the principal hormone of the luteal phase and prepares the endometrium for the implantation of the fertilized ovum. If the **ovum is not fertilized**, the corpus luteum regresses and **plasma ovarian hormone concentrations fall;** the menstrual cycle takes its course, with sloughing of the endometrium and menstrual bleeding.
- As the plasma **ovarian hormone** concentrations fall, the concentrations of **LH and FSH** in plasma begin to **rise** and the cycle recommences. If fertilization does occur, pregnancy may supervene.


(A) Gonadotropins
(from anterior pituitary)
Luteinizing
hormone (LH)
Follicle-stimulating hormone (FSH)
(B) Events in ovary
Egg
Developing follicle
Ovulation
Corpus luteum
(C) Ovarian hormones
Estrogen
Progesterone
(D) Uterine lining
Menses
Follicular phase
Luteal phase
0
5
10
15
20
25
Day of menstrual cycle

Normal gonadal function

- Interpretation of plasma sex hormone concentrations must be made in relation to the stage of the cycle.
- The plasma **progesterone concentration** should be measured in a blood sample taken during the second half of the menstrual cycle; **usually day 21**.
- A value within the reference range for the time of the cycle is good probable evidence of ovulation, whereas a value in the range expected in the follicular phase indicates the absence of a **corpus luteum,** and therefore of **ovulation**.
- **Ovulation** may also be detected by **ultrasound** examination of the ovaries.
- **Progesterone** secretion is associated with a **rise in body temperature,** which may be monitored serially to determine the time of ovulation

The menopause

- The menopause is defined as **the time of permanent cessation of menstruation, the average age being about 50 years.**
- The menopause occurs when all **the follicles have atrophied**.

- **Plasma concentrations of estrogens** fall and those of **FSH** and, to a lesser extent, **LH increase** after removal of the negative feedback to the pituitary.
- These findings are therefore similar to those of **primary gonadal failure.**

- Diagnosis of the menopause is a clinical one, although a plasma **FSH** consistently greater than **20–40 IU/L is suggestive of ovarian failure** but not a guarantee of sterility, and thus suitable contraception is necessary for 1 year of amenorrhoea in patients over 50 years and for 2 years in those under 50 years.

- It may sometimes be useful to measure **plasma estradiol concentrations** in women with estradiol HRT implants, as very high levels may be associated with tachyphylaxis, that is, tolerance to dose. There may also be a place for its measurement in women on transdermal estradiol HRT to ensure adequate absorption.

The male

- Development of male characteristics
- In the presence of the Y chromosome the fetal gonads develop into testes at **about 7 weeks' gestation.**
- The **Sertoli cells** secrete anti-Müllerian hormone that inhibits the Müllerian duct (the female oviduct), in the male embryo.
- The intracellular conversion of testosterone to dihydrotestosterone by 5 α reductase is essential for the development of male external genitalia.
- If this enzyme is deficient, varying degrees of feminization may occur, causing male **pseudo hermaphroditism**.

Male puberty

- During childhood, the rate of secretion of gonadotrophins from the anterior pituitary gland is low.
- As puberty approaches, the pulse amplitude and frequency of LH secretion increase. Initially, this occurs during sleep, but later continues throughout the day.
- **Leydig cell function and testosterone secretion** increase and stimulate the development of secondary male characteristics.
- **Gonadotrophin secretion** also stimulates **meiosis** of previously dormant germ cells in the seminiferous tubules, and thus the production of sperm.
- **Deficient secretion** of either pituitary or gonadal hormones may cause **delayed puberty.**
- The male germ cells produce spermatozoa continuously after puberty.
- **Spermatogenesis** is dependent on both **normal Sertoli cell function** and **testosterone secretion** by Leydig cells. Therefore both **LH and FSH** are needed for normal spermatogenesis, but testosterone secretion can occur in the absence of normal seminiferous tubules.

Disorders of Gonadal Functions

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Disorders of gonadal functions in females

Gonadal dysfunction in women usually presents with any or all of the following:

- amenorrhea,
- hirsutism,
- virilism,
- infertility.

AMENORRHEA

- **Amenorrhea** is defined as the absence of menstruation; it may be due to hormonal abnormalities.
- If there is **ovarian failure**, pituitary gonadotrophin concentrations in plasma are high (hypergonadotrophic hypogonadism);
- if the cause is in the **hypothalamus or anterior pituitary** gland, gonadotrophin secretion is reduced (hypogonadotrophic hypogonadism).
- **Amenorrhea** may be classified as either **primary or secondary.**

Primary amenorrhea

- occurs when the patient has **never menstruated** and is most commonly associated with **delayed puberty**.
- The age of the **menarche is very variable**.
- Extensive investigation should probably be postponed until around the **age of 16**, unless there are other clinical features of either **endocrine disturbances**:
- hirsutism
- virilism
- chromosomal abnormalities **Turner's syndrome** (45,X0).

Secondary amenorrhea

- occurs when previously established menstrual cycles have stopped and is **most commonly** due to physiological factors such as ***pregnancy*** or the ***menopause***.
- Other causes include severe illness, excess or rapid weight loss for any reason, including anorexia nervosa, or stopping oral contraceptives.
- These should be considered before extensive and potentially dangerous investigations are started.
- A number of **endocrine disorders**, such as **hyper prolactinaemia, hypothyroidism, Cushing's syndrome and acromegaly**, may present with **amenorrhea**.

Hirsutism and virilism

- Increased plasma free **androgen concentrations**, or increased tissue **sensitivity to androgens**, produce effects ranging from increased *hair growth* (hirsutism) to marked *masculinization*, with virilism.
- **Testosterone** is the most important androgen.
- **In typical women** about **half the plasma testosterone** comes from the **ovaries**, both by **direct secretion** and by **peripheral conversion of androstenedione**.
- **The rest** is derived from **adrenal cortex** by *direct secretion or peripheral conversion of adrenal androgens, androstenedione and dehydroepiandrosterone (DHEA).*
- Because of the extensive interconversion of androgens, the source of a slightly raised plasma testosterone concentration may be difficult to establish.
- In general, markedly raised plasma concentrations of DHEA or its sulphate (DHEAS) indicate an adrenocortical origin.

- The **biological activity of testosterone** depends on the plasma free hormone concentration.
- The plasma total concentration is also influenced by the concentration of the binding protein SHBG.

Increased SHBG	Decreased SHBG
Oestrogens	Androgens
Hyperthyroidism	Hypothyroidism
Liver disease	Obesity
Catabolic states	Protein-losing disorders
Human immunodeficiency virus (HIV)	Undernutrition
Anticonvulsants	Glucocorticoids, anabolics, progestins

HIRSUTISM

- is defined as an excessive growth of hair in a male distribution and is common, possibly occurring in 10% of women.
- A common cause is **familial or racial hirsutism**.
- For example, some *Mediterranean women* have more terminal hair, and fair-skinned *Europeans* have the least.
- This difference may be due to racial differences in **5-α-reductase activity** in skin.
- **Plasma testosterone concentrations** may be slightly raised, but are often within the female reference range.
- A **raised testosterone** concentration, **particularly above 5 nmol/L**, may indicate a tumor of the adrenal or ovary, which must be excluded.
- However, the plasma concentration of **free hormone** may be significantly **increased** if that of *SHBG is low*.
- A raised plasma **17-hydroxyprogesterone** concentration may indicate CAH.

Some causes of hirsutism are

- Idiopathic
- Racial
- Polycystic ovary syndrome
- Cushing's syndrome
- Congenital adrenal hyperplasia
- Ovarian tumors
- Adrenal tumors
- Exogenous androgens and drugs with androgenic activity

VIRILISM

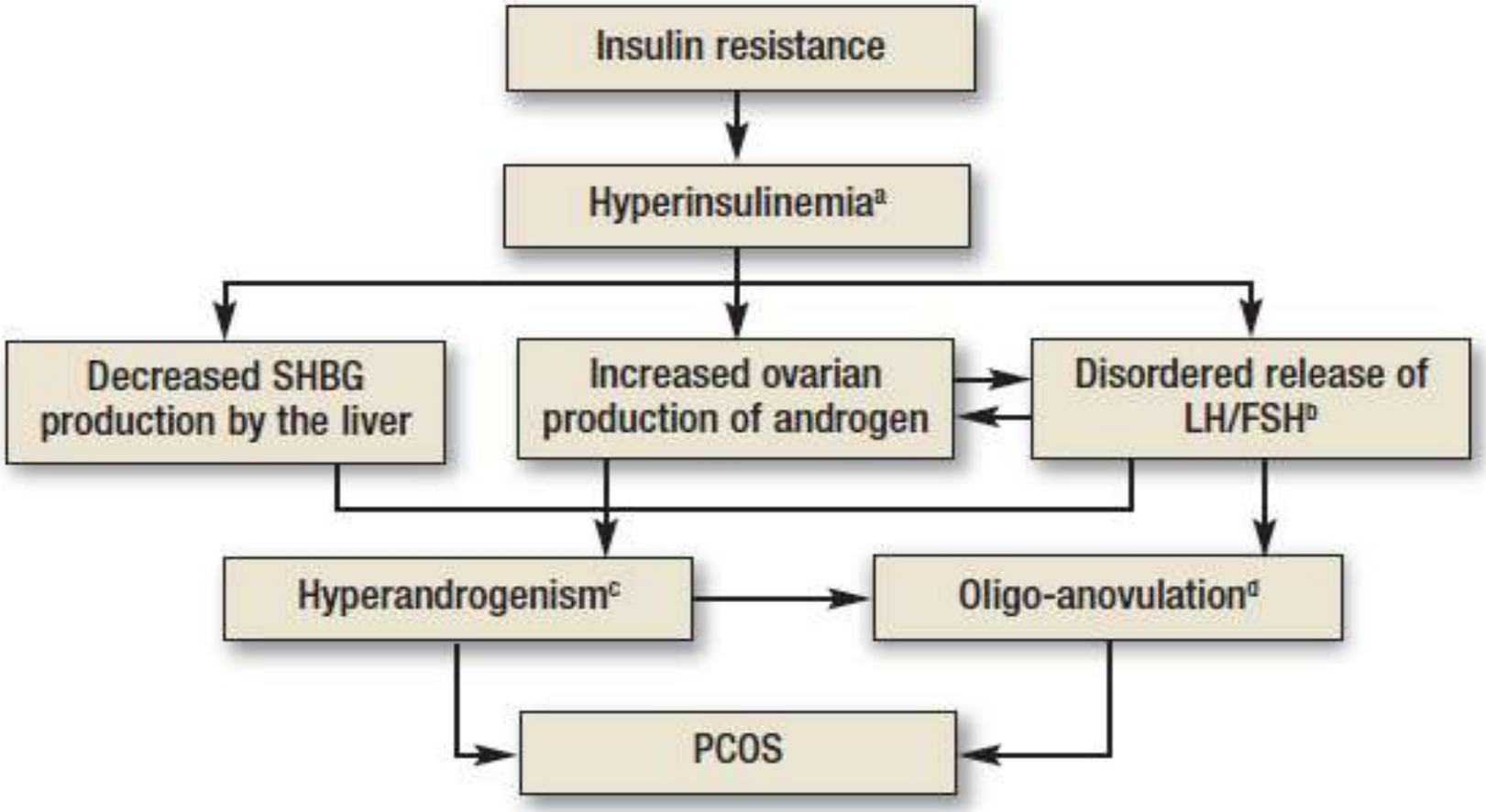
- is characterized by additional evidence of excessive androgen secretion such as an **enlarged clitoris** (clitoromegaly), increased **hair growth of male distribution**, receding **temporal hair**, **deepening** of the **voice and breast atrophy**.
- It is always associated with increased *plasma testosterone* concentrations.
- Plasma *DHEA and DHEAS* concentrations may also be increased.
- **Virilism** usually implies an *adrenal or ovarian androgen* source and can be a cause of female pseudohermaphroditism.

Some causes of virilism are:

- Idiopathic
- Ovarian tumours that secrete androgens, mainly testosterone
- Adrenocortical disorders, e.g. carcinoma or congenital adrenal hyperplasia
- Cushing's syndrome
- Exogenous androgens or progestogens

PCOS (Leventhal–Stein syndrome)

- This is a condition showing features of **hyperandrogenism** with **anovulation** and **abnormal ovarian morphology** and is the most common cause of **anovulatory infertility**.
- Presenting clinical symptoms may also include **hirsutism**, **menstrual disturbances**, **enlarged polycystic ovaries** (may be demonstrated on ultrasound scanning of the ovaries), and **infertility**.
- Plasma *testosterone and androstenedione* concentrations are often increased.
- The plasma LH may be elevated with normal FSH.
- Because *plasma SHBG* concentrations are reduced in **obese** individuals, the plasma concentration of *free testosterone* is often increased. The plasma *prolactin* concentrations may also be high.
- PCOS is also associated with *insulin resistance*, *obesity* and elevated *plasma insulin concentrations*, which may **stimulate** *androgen production* from the **ovarian thecal cells**. Individuals may also have *hyperlipidaemia*, *glucose intolerance* and *hypertension*.



[a]Elevated levels of insulin in the blood.
[b]Pituitary hormones that stimulate the ovaries.
[c]Elevated blood levels of male hormones.
[d]Absent or irregular ovulation.

LH indicates luteinizing hormone; FSH, follicle-stimulating hormone; PCOS, polycystic ovarian syndrome; SHBG, sex hormone–binding globulin.

DISORDERS OF GONADAL FUNCTIONS IN MALES

- **Gonadal dysfunction in men** may present with the symptoms of androgen deficiency or of infertility, **or both**.

1- Androgen deficiency is the result of impaired testosterone secretion by Leydig cells.

- The patient may present with delayed puberty or with regression of previously established male characteristics that are dependent on testosterone (hair distribution, potency and libido).
- There may be *primary testicular dysfunction*, in which case the low plasma testosterone concentration is accompanied by raised plasma LH concentrations (**hypergonadotrophic hypogonadism**).
- *Dysfunction secondary to pituitary or hypothalamic* disease conversely results in low plasma LH concentrations (**hypogonadotrophic hypogonadism**).

DISORDERS OF GONADAL FUNCTIONS IN MALES

- **2- Infertility** may be caused by *androgen deficiency*, but most infertile men have normal plasma androgen concentrations.
- **Sertoli cell function** is dependent on b*oth FSH and testosterone produced locally*.
- **Semen analysis** is an important investigation for male infertility. However, **biochemical tests** may sometimes help; if the cause is primary testicular failure, the plasma *testosterone concentration is low*, and *reduced inhibin* production by **Sertoli cells** causes a *rise in FSH* concentrations.
- If there is evidence of a **failure of spermatogenesis**, the plasma FSH concentration should be measured.
- If the cause is *secondary to anterior pituitary failure*, both plasma **testosterone and FSH concentrations are low**.
- **Hyperprolactinaemia** is much less common in males than in females, but its presence may indicate a *pituitary tumor.* Chromosomal abnormalities such as **Klinefelter's syndrome** (47,XXY) may also cause abnormal male gonadal function.

GYNAECOMASTIA

- This is the **enlargement of male breast tissue** (glandular and **not adipose** tissue), which can be *unilateral or bilateral*.
- The imbalance between **estrogen action relative to androgen action** at the breast tissue level appears to be the main etiology of gynecomastia.
- An increase in the **estrogen to androgen ratio**, can be seen physiologically at *puberty or in the elderly*.
- **Elevated serum estrogen levels** may be a result of *estrogen-secreting neoplasms* or their precursors (*Leydig or Sertoli cell tumors*, [*hCG]–producing tumors*, and *adrenocortical tumors*) but more commonly are caused by **increased extragonadal** conversion of androgens to estrogens by *tissue aromatase* **(as occurs in obesity).**

- Levels of **free serum testosterone** are decreased in patients **with gonadal failure**, which can be *primary (Klinefelter's syndrome)* or *secondary (hypothalamic and pituitary disease).* **Androgen resistance syndromes** due to impaired activity of enzymes involved in the **biosynthesis of testosterone can also be associated with gynecomastia.**

- The balance between **free testosterone and estrogen** is also affected by serum levels of SHBG, which is the proposed mechanism of gynecomastia in certain conditions, such as hyperthyroidism, chronic liver disease, and the use of some medications such as spironolactone. **Receptors of androgens** can also have **genetic defects** or **become blocked** by certain medications, and the **receptors of estrogens can be activated** by certain medications or environmental exposures.

OTHER DISORDERS OF REPRODUCTIVE ORGANS

Precocious puberty

- This can be defined as the appearance of secondary sexual characteristics **before the age of 8 years** in either females or males. It can be:
- True (central) precocious puberty, which is gonadotropin-dependent, is the early maturation of the entire hypothalamic-pituitary-gonadal axis, with the full spectrum of physical and hormonal changes of puberty. **It is caused by cerebral tumors, infection or trauma, or hypothyroidism**.
- Pseudo precocious puberty is much less common and refers to conditions in which increased production of sex steroids is gonadotropin-independent. **It is caused by adrenal or ovarian/testicular tumors**

OTHER DISORDERS OF REPRODUCTIVE ORGANS

Ambiguous genitalia and intersexuality

- During the **fetus's second month**, its indifferent gonad is stimulated to develop into a testis, initiated by testis-determining factor derived from the sex-determining region of the Y chromosome (SRY) gene.

- If this region is absent or abnormal, the indifferent gonad develops into an ovary. Development of male external genitalia is mediated by testosterone, which is converted to dihydrotestosterone by 5 α reductase

Female pseudohermaphroditism

- Individuals with this condition usually show XX chromosomes but male genitalia characteristics and virilism.
- More commonly, it can be caused by high concentrations of maternal androgens during pregnancy or increased concentrations of fetal androgens, such as in CAH, including deficiency of 21-hydroxylase or 11-hydroxylase, which result in increased endogenous testosterone production

Male pseudohermaphroditism

- Individuals with this condition usually have chromosomes XY and possess two testes but female external genitalia.
- Causes include CAH such as 17α hydroxylase deficiency.
- Other causes are deficient testosterone biosynthesis and androgen receptor defects (testicular feminization or 5 α reductase deficiency).

True hermaphroditism

- This is very rare, with individuals having both testicular and ovarian tissue.

BIOCHEMICAL INVESTIGATION OF GONADAL DISORDERS

- In suspected cases, a careful clinical history, including medications, and physical examination can be useful.
- Measurement of **plasma LH, FSH, TSH, prolactin, estradiol, testosterone and SHBG** may reveal a diagnosis.
- If intersex conditions are sought, **karyotyping** may be useful.
- Sometimes the more specialized tests described below are necessary

GONADOTROPHIN-RELEASING HORMONE TEST

Gonadotrophin-releasing hormone (a decapeptide) released by the hypothalamus stimulates the release of the gonadotrophins LH and FSH from the normal anterior pituitary gland. The test is used to diagnose hypothalamic–pituitary disease in precocious and delayed puberty with low basal gonadotrophin concentrations.

IV injection of **100 µg of GnRH** is given. Plasma **LH and FSH** concentrations are measured in blood drawn *before and at 20 and 60 min after the injection.*

Note: as with any test, administration of an exogenous peptide may induce an allergic reaction in some patients, and therefore resuscitation facilities should be at hand and the test should be performed by experienced staff. Sometimes nausea and abdominal pain are experienced.

GONADOTROPHIN-RELEASING HORMONE TEST

- The test can sometimes be combined with the **TRH** and **insulin or glucagon stimulation tests** as part of the triple pituitary test, although this is now rarely performed.
- In **pre pubertal children**, plasma LH and FSH are usually both less than 2.0 U/L.
- In normal subjects, the levels of **plasma LH and FSH** at least double from their basal levels at 20 min, but this rise fails to occur in patients with pituitary hypofunction.
- Conversely, an **exaggerated response** may be seen in patients with hypothalamic disease

HCG STIMULATION TEST

HCG shares a common subunit with LH and stimulates testicular Leydig cells to release androgens. It has a long half-life and elicits a rise in plasma testosterone after 72–120 h.

The test may be indicated in the following situations:

- to confirm the presence of testes,
- in infants with ambiguous genitalia and palpable gonads,
- in males with delayed puberty,
- in some cases of undescended testicles.

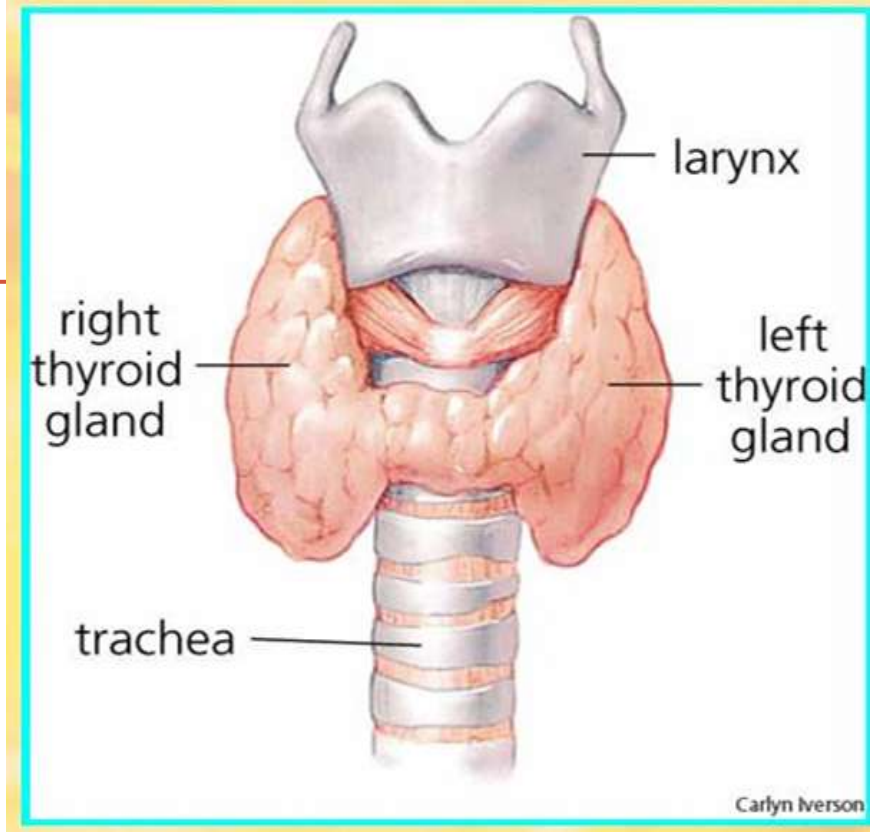
On day 0, blood is taken for testosterone. Then 2000 hCG units are given on days 0 and 2 by intramuscular injection. On day 4, blood is again taken for testosterone, androstenedione and dihydrotestosterone.

Normally there is at least a two-fold increase in testosterone concentration, but in the absence of testes there is no testosterone response

THYROID GLAND &DISORDERS

lecture in Clinical chemistry

2018-2019



Thyroid Gland

- Located in middle anterior part of neck: below larynx, in front of trachea
- "Butterfly" shape
- 2 lobes connected by isthmus
- ↑ in size : puberty & pregnancy
- Rich blood supply: able to deliver high levels of hormones in short period of time
- Produces Thyroxin (T4) & Tri-iodothyronine (T3)
- Calcitonin : involved in calcium & phosphate homeostasis

Thyroid gland

- **Pituitary TSH synthesis** and release are stimulated by **TRH,** produced in the hypothalamus .
- The **action of TRH** can be over-ridden by high circulating free **T4**
- **The exogenous TRH** has little effect on TSH secretion in hyperthyroidism .
- **Thyroid hormones** reduce TSH secretion by negative feedback.
- **T3** binds to **anterior pituitary nuclear receptors** , most of the intracellular T3 is derived from circulating f T4.
- The gland is more sensitive to changes in **plasma T4** than to T3 concentrations.

Synthesis

- Thyroid hormones are synthesized in the thyroid gland .
- The iodination and coupling of two molecules of the amino acid tyrosine
- **Iodide in the diet** is absorbed rapidly from the small intestine. **Sea foods and iodized salt** are the main dietary sources of iodide. Normally about a **third of dietary iodide** is taken up by the thyroid gland and the rest is renal excreted.
- Iodide **is actively taken up** by the thyroid gland under the control of (TSH) via a sodium/iodide symporter. Uptake is blocked by **thiocyanate and perchlorate.**
- The **concentration of iodide** in the gland is at least 20 times that in plasma and may exceed it by 100 times or more.

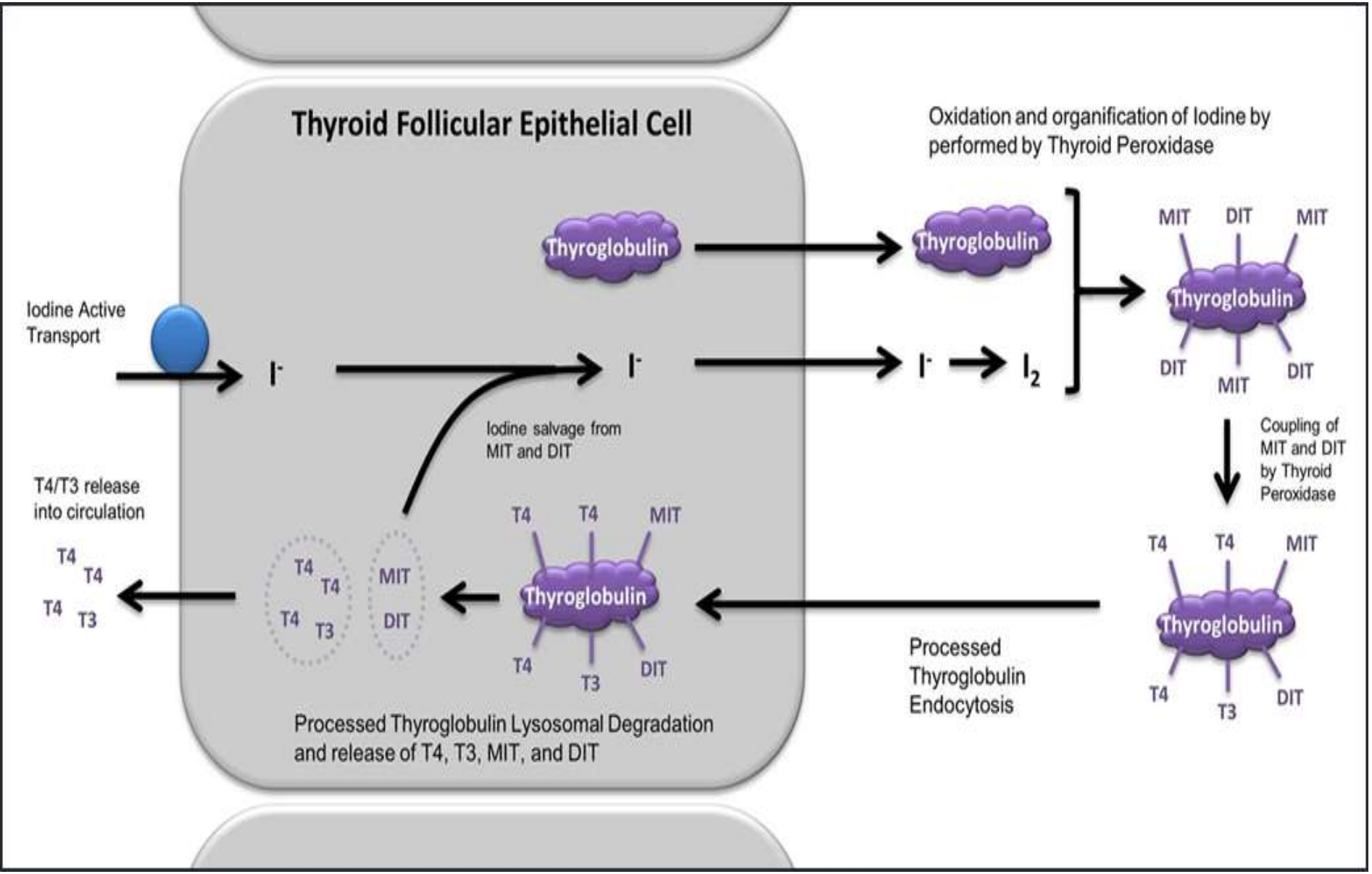
Synthesis

- **Iodide converted to iodine** within the thyroid gland, catalysed by thyroid peroxidase (TPO). **Iodination of tyrosine residues** in a glycoprotein to form mono-iodotyrosine (MIT) and di-iodotyrosine (DIT) mediated by the enzyme TPO.

  This step is inhibited by carbimazole and propylthiouracil.

- **Iodotyrosines** are coupled to form T4 (DIT& DIT) and T3 (DIT& MIT) (stored in the lumen of the thyroid follicular cells). Normally much more **T4 than T3 is synthesized.** If there is an inadequate supply of iodide, the **ratio of T3 to T4** in the gland increases.

- The **thyroid hormones**, thyroglobulin is taken up by the follicular cells, by a *endocytosis and then phagocytosis*, and T4 and T3 are released by **proteolytic enzymes** into the bloodstream , this process is stimulated by TSH and inhibited by iodide.


Thyroid Follicular Epithelial Cell
Oxidation and organification of Iodine by performed by Thyroid Peroxidase
Thyroglobulin
Thyroglobulin
MIT DIT MIT
Thyroglobulin
DIT MIT DIT
Iodine Active Transport
I-
I-
I-
I2
Iodine salvage from MIT and DIT
Coupling of MIT and DIT by Thyroid Peroxidase
T4/T3 release into circulation
T4 T4 T4 T3
T4 T4 T4 T3
MIT DIT
T4 T4 MIT
Thyroglobulin
T4 T3 DIT
T4 T4 MIT
Thyroglobulin
T4 T3 DIT
Processed Thyroglobulin Endocytosis
Processed Thyroglobulin Lysosomal Degradation and release of T4, T3, MIT, and DIT

Thyroid hormones synthesis

- **Thyroid hormones** are immediately bound to plasma proteins.
- **MIT& DIT** release are de-iodinated to reuse iodine again.
- **Each step** is controlled by specific enzymes, and **congenital deficiency** of any of these enzymes can lead to **goiter** and if severe, **hypothyroidism**.
- The **uptake of iodide** and the **synthesis** and **secretion** of thyroid hormones, is regulated by **TSH** .
- About 10 times **more T4 than T3** is formed. **Most of the T3** being formed by de-iodination in the **liver**, **kidneys and muscle**.
- Most of the plasma T4 and T3 are **protein bound**.

Protein binding

- 70% thyroxine-binding globulin (TBG),15% transthyretin, about10–15% bound to albumin.
- the **free fraction** is the physiologically active form, which also ***regulates TSH secretion*** .
- Changes in the plasma concentrations of the binding proteins, alter **plasma total T4 and T3 concentrations**, but not the concentrations of free hormones.
- About **80% of the plasma T3** is produced by the removal of an iodine atom from the outer (b) ring; The **remaining 20%** is secreted by the thyroid gland. De-iodination of the inner (a) ring produces **reverse T3** (Inactive) . **T3** binds more avidly to thyroid receptors than T4 and is the main active form.

Level of hormones

The conversion of T4 to T3 may **be reduced** by many factors:

– systemic illness,

– prolonged fasting,

– drugs such as β -blockers (propranolol) or amiodarone (200 mg of this anti-arrhythmic drug contains about 75 mg of iodine);

The conversion of T4 to T3 may **be increased** by hepatic enzyme induce such as phenytoin.

T3

- **The plasma T3 concentration** is a poor indicator of thyroid hormone secretion (it is influenced by many non-thyroidal factors)
- Its measurement is rarely indicated, except if **thyrotoxicosis** is suspected. Normal plasma **concentrations of T3** are very low.

- **In hyperthyroidism**, the increase in plasma T3 or fT3 concentrations is greater, and usually occurs earlier than that of T4 or fT4. **Occasionally in hyperthyroidism** the plasma T3 or fT3 concentrations are elevated but not those of T4 or fT4 (T3 toxicosis)

Action of thyroid hormones

Thyroid hormones:

1- Affect many metabolic process, increasing oxygen consumption.

2- Bind to specific receptors in cell nuclei and change the expression of certain genes.

3- Essential for normal growth, mental development and sexual maturation

4- Increase the sensitivity of the CVS and CNS to catecholamine, thereby influencing cardiac output and heart rate.

T4

- Plasma T4 is more than 99% protein bound; **Plasma total T4** assays reflect the protein bound rather than the free hormone fraction. Total T4 reflects fT4 concentrations, unless there are abnormalities of binding proteins.

- In the **euthyroid state,** about a **third** of the binding sites on TBG are **occupied** by T4 and the **remainder are unoccupied**, irrespective of the concentration of the binding protein.

- **In hyperthyroidism**, both *plasma total and fT4 concentrations* are increased and the number of unoccupied binding sites on TBG is decreased.
- **In hypothyroidism**, the opposite of the above occurs.

T4

- An **increase in plasma TBG** concentration causes an **increase** in both bound T4 and unoccupied binding sites but no change in plasma fT4 concentrations.

an increase may occur in:

- a high **estrogen concentration** during pregnancy or in the newborn infant,
- **estrogen therapy**, for example certain oral contraceptives or hormone replacement therapy,
- **inherited TBG excess** (rare).

T4

A **decrease in plasma TBG concentration** decreases both bound T4 concentrations and unoccupied binding sites, but does not alter the plasma fT4 concentration

Causes:

- *Severe illness* (temporary),
- *Loss of low Mwt proteins* usually in the urine as in nephrotic syndrome,
- *Salicylates, androgens or danazol* treatment; bind to TBG and displace T4
- *Inherited TBG* ***deficiency*** (rare)

Thyrotrophin-releasing hormone test

- The **TRH test** is used to confirm the diagnosis of *secondary hypothyroidism*, or occasionally to diagnose early *primary hypothyroidism*.
- It is rarely used to diagnose *hyperthyroidism*,
- Have a place in the ***differential diagnosis*** of thyroid resistance syndrome or TSH-secreting pituitary tumors (**TSH-omas**).

TRH test

- In normal subjects, **plasma TSH concentration** increases at 20 min by and exceeds the upper limit of the reference range, with a small decline at 60 min.
- An exaggerated response at 20 min and a slight fall at 60 min are suggestive of **primary hypothyroidism.**
- A **normal or exaggerated increment** but delayed response, with plasma TSH concentrations higher at 60 min than at 20 min, suggests **secondary hypothyroidism**.
- If clinically indicated, pituitary and hypothalamic function should be investigated.

TRH stimulation test

- A **flat response of TSH** of less than 5 mU/L is compatible with *primary hyperthyroidism*, although this may also occur in some *euthyroid patients with multinodular goitre*.
- Interference of assays by **immunoglobulins** can cause a spurious elevation of T4 or T3 (**free hormones**), respectively, when assayed by immunoassay.
- This needs to be remembered when interpreting thyroid function test results

Hypothyroidism

- Suboptimal circulating concentrations of thyroid hormones. More prevalent with age, more common in women. May develop insidiously, in early stages may cause only vague symptoms.

Signs& Symptoms

- There is a generalized slowing down of metabolism, with lethargy, bradycardia, depression and weakness. the patient may present with weight gain, myopathy, menstrual disturbances, and constipation. dry skin, the hair may fall out and the hoarse voice. Subcutaneous tissues are thickened (pseudo-edema) with a histological myxoid appearance (myxedema)

- **In severe cases**, coma with profound hypothermia may develop. the clearance of plasma (LDL) cholesterol **is impaired** and plasma cholesterol concentrations may be **moderately high**.

- **Plasma CK activity** is often raised , due to myopathy.

Hypothyroidism

- **Hyponatraemia** very rarely present in patients with profound hypothyroidism or myxoedema coma caused by **increased ADH** release with excessive water retention, occasionally worsened by a constrictive pericardial effusion that some patients develop.

- May be associated with **hyperprolactinaemia**. Reduced plasma **(SHBG)** concentration.
- A **macrocytic anemia** may be observed, with raised *mean corpuscular volume* (MCV).
- In **severe hypothyroidism** a reduced GFR due to impaired renal perfusion.
- The most common cause worldwide is iodine deficiency. In areas of adequate iodine intake, **acquired type** is mainly due to autoimmune thyroiditis or Hashimoto's thyroiditis, which (more frequently in women and the elderly).

- About **90% of patients** have positive thyroid antibodies, anti-thyroid peroxidase (anti-TPO), anti-thyroglobulin (anti-Tg) TSH receptor blocking antibodies). There may also be a **goiter**; may be associated with other *autoimmune diseases such as type 1 DM, adrenal insufficiency and pernicious anemia.*

Hypothyroidism

- **Rare causes of primary type** are exogenous goiterogens (substances that interfere with thyroid iodine uptake and thus can result in a goiter) dyshormonogenesis, inherited deficiencies of any of the enzymes involved in thyroid hormone synthesis, may present in childhood.

- **In most cases**, prolonged TSH stimulation, due to reduced negative feedback, causes goiter. **The most common form is due to failure to incorporate iodine into tyrosine.**

The **perchlorate discharge test** useful to diagnose iodination and trapping defects (is rarely used).

- **Secondary type** due to low concentrations of TSH or to hypothalamic TRH deficiency. Much less common than primary hypothyroidism.
- In *long-standing secondary hypothyroidism*, the thyroid gland may **atrophy irreversibly**. The essential biochemical difference between primary and secondary hypothyroidism is in the **plasma TSH** concentration, which is high in the former and inappropriately low in the latter

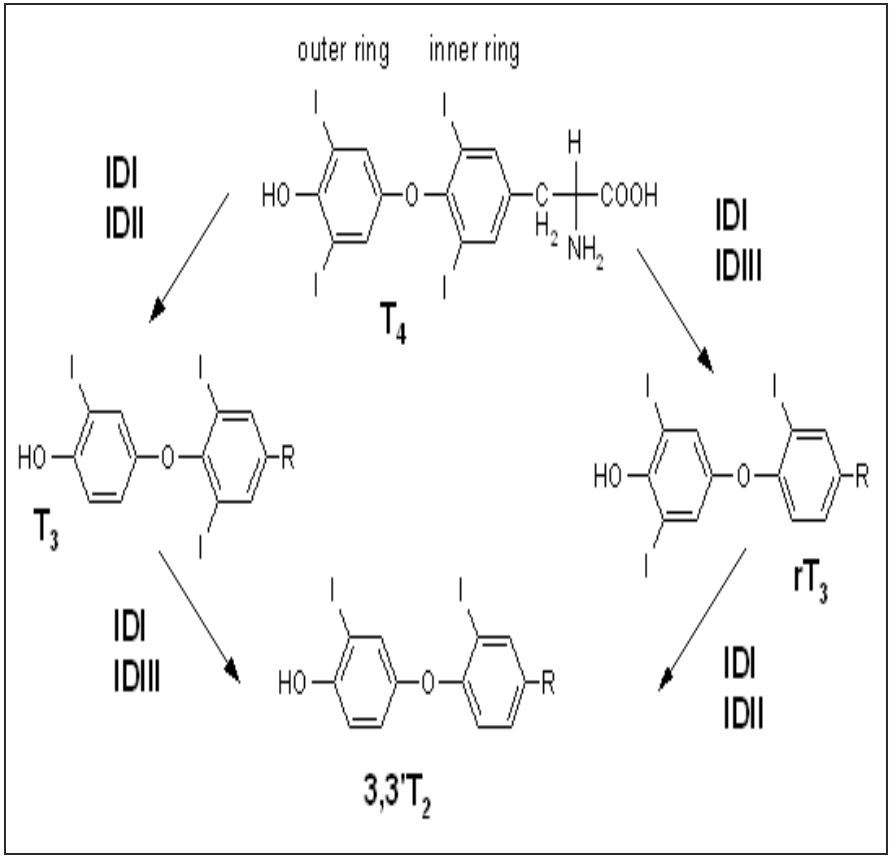
Hypothyroidism

Very rarely a ‘consumption’ hypothyroidism (**extensive haemangioma**) which contains iodothyronine deiodinase. This *selenoenzyme* catalyzes the conversion of T4 to *reverse T3* and the conversion of T3 to *3,3¢- DIT*, both of which are **biologically inactive.**

Pregnancy and neonatal hypothyroidism

0.3% of pregnancies the mother has Hashimoto’s disease.

Thyroid hormones are essential for fetal development (treating the mother with thyroxine).



Treatment of hypothyroidism

- Usually with T4,titrated until the plasma TSH is within the reference range.
- **Plasma TSH concentrations** may not adequately reflect tissue hypothyroidism, better to be guided by *plasma fT4* concentrations and clinical features.
- On rare occasions, such as in **hypothyroid comas**, *T3* is given instead(its action is more immediate).
- The **response to T4** therapy checked **every 2-3 m** until the patient is stable, after which **6-12 m** blood checks may be useful.

Treatment of hypothyroidism

- **Thyroxine** used with caution in patients with *ischemic heart disease* (fear of worsening **angina pectoris**),so low doses initially plus B-blockers indicated.
- **TSH assays** are of little value in monitoring secondary hypothyroidism ;fT4 is better.
- **Thyroxine** therapy may precipitate an **Addisonian crisis** in patients with concomitant ***adrenal insufficiency***. Overtreatment with T4 can induce **atrial fibrillation and osteoporosis**;**TSH** concentrations are often **low or suppressed.**
- **If a patient** is non-compliant with treatment and only takes T4 near to **the time of (TFT)** → a high plasma TSH with high plasma fT4 concentrations. There is **insufficient T4** to normalize **plasma TSH**, and yet the **high plasma fT4** reflects the **recent taking of T4**.

Subclinical hypothyroidism

- Subclinical (**compensated hypothyroidism**) plasma TSH is ↑ ,total or fT4 within the reference range. 10 % individuals over the **age of 60 years**
- Some patients have positive thyroid antibodies (**anti-TPO or anti-Tg)**,
- Each year about **2–5% of thyroid antibody-positive patients** go on to develop **hypothyroidism**.
- Some patients **asymptomatic**, some have **symptoms**
- **Thyroxine therapy** may be indicated particularly in *pregnancy*, *symptomatic patient*, or with *positive thyroid antibodies* and *plasma TSH more than10 mU/L*.
- It can be associated with increased **risk of cardiovascular disease**.

Thyroid hormone resistance

- Plasma **total T4 and fT4 concentrations** are elevated with **normal or slightly raised TSH concentration**.
- **Some patients** appear *euthyroid*, but others may present with *hypothyroid symptoms*,
- Defect may inherited as an **Autosomal dominant trait** in some patients. Thought to be due to a **defect in T4 and/or T3 receptors** and may be associated with other **end-organ resistance states**

Lab investigation

- A **careful history (including drugs) ,checking for a goiter.**
- **Plasma TSH and total T4 or fT4 concentrations** should be measured.
- Slightly **elevated plasma TSH and normal fT4** concentrations suggest **compensated hypothyroidism**,
- **Measuring circulating thyroid antibodies** may be useful, that is, anti-TPO tests should be repeated **after 3–6 m** as some patients may develop **full blown hypothyroidism**.
- **Raised plasma TSH and low fT4 concentrations** suggest primary hypothyroidism, **thyroid antibodies** should be measured and if **positive** *other autoimmune diseases excluded*.
- **Low plasma TSH and low fT4 concentrations** may indicate hypothalamic or pituitary disorder.
- **TRH test** should be done, and *pituitary gland assessed* .
- *Raised plasma TSH* and *raised/normal plasma fT4* in the presence of hypothyroid symptoms may **indicate thyroid hormone resistance**.

Hyperthyroidism (thyrotoxicosis)

- Sustained high **plasma T4 and T3**. Generalized increase in the metabolic rate clinically heat intolerance, a fine tremor, tachycardia including atrial fibrillation, weight loss, tiredness, anxiety, sweating and diarrhea.

Biochemical features :

- *Hypercalcaemia* (occasionally with severe thyrotoxicosis) , increased turnover of bone cells(direct action of thyroid hormone).
- *Hypocholesterolaemi*a can occur, due to increased LDL clearance.
- *Hypokalaemia* may occur, associated with hyperthyrotoxic periodic paralysis.
- *Plasma SHBG* is increased.
- *Plasma CK* may be increased with thyrotoxic myopathy

Graves' disease

The most common form occurs **more in females** caused by autonomous secretion from a diffuse goiter characterized by:

- **Exophthalmos**, due to lymphocytic infiltration and swelling of retro-orbital tissues of the eyes,
- Sometimes **localized thickening** of the subcutaneous tissue over the shin (pretibial myxedema).
- **Autoimmune circulating antibodies** including anti-TPO, associated with other autoimmune diseases ***such as type1DM, adrenal insufficiency and pernicious anemia***.

Graves' disease Detection

- Thyroid antibodies are detectable in some cases, such as **thyroid-stimulating immunoglobulin** (TSI), which is directed *towards epitopes of the TSH receptor* and acts as a *TSH receptor agonist.*

- **Nuclear medicine tests** may show *a high radioactive uptake* of iodine by the thyroid gland.

Sub acute thyroiditis

- A destructive thyroiditis resulting in the release of preformed thyroid hormones; associated with extremely elevated thyroid hormones and no radioactive iodine uptake by the thyroid gland .

Three subtypes

- **Granulomatous or painful**,
- **Lymphocytic or silent painless**,
- **Post-partum**.

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The clinical course progresses through 6-8 w **of thyrotoxicosis** 2-4 m of **hypothyroidism** and a return to euthyroidism in about 90% of patients.

- The **painful or granulomatous** is a **viral disease** and is associated with human leucocyte antigen (HLA)-Bw35.
- The **lymphocytic and post-partum** are **autoimmune**
- Treatment is **supportive** (self-limiting)

Toxic nodules

- Either *single or multiple*,
- In a **nodular goiter** may secrete thyroid hormones autonomously.
- The secretion of TSH is suppressed by negative feedback,
- The nodules may be detected by *their uptake of radioactive* iodine or technetium, with suppression of uptake in the rest of the thyroid tissue '**hot nodules**'.
- **Most commonly in older patients**, who may present with only one of the features of hyperactivity, usually cardiovascular symptoms such as atrial fibrillation.
- ***Toxic multinodular*** goiter is also called **Plummer's disease**.

Rare hyperthyroid states

- **Jod–Basedow syndrome** in patients with excess iodide intake, from the diet or from iodine-containing contrast medium. High iodine intake may be assessed by urinary iodide assay.

- **Metastatic thyroid carcinoma** can produce thyroid hormones. In **struma ovarii**, ectopic thyroid tissue is found in dermoid tumors or ovarian teratomas.
- Patients with **choriocarcinoma** or **molar hydatidiform** pregnancy have extremely high concentrations of β-HCG that can activate the **TSH receptor.**
- Rarely, the **pituitary tumor** releases TSH, resulting in thyrotoxicosis.

Pathophysiology of hyperthyroidism

- **Plasma T4 or fT4 and T3 and fT3** concentrations are increased .
- **Much of the T3** is secreted directly by the thyroid gland, and the increase in plasma T3 concentrations is greater, and usually evident earlier, than that of T4.
- Rarely, only **plasma T3 and fT3 concentrations** are elevated (T3 toxicosis).
- In both situations, **TSH secretion** is suppressed by negative feedback, and plasma TSH concentrations are either *very low or undetectable*

Treatment of hyperthyroidism

- The etiology must investigated .
- the selection of treatment depends on the cause, the clinical presentation and the age of the patient.
- β-blocker drugs such as propranolol **inhibit the peripheral conversion of T4 to T3**, may be used initially.
- Carbimazole **inhibits the synthesis of T3 and T4**;
- Propylthiouracil additionally **inhibits T4 to T3 conversion**.

Some clinicians use block-and-replace regimens:

Carbimazole is used to ‘block’ thyroid secretion, and concurrent exogenous T4 maintainsT4

Treatment

- Carbimazole have **lethal side effect** of bone marrow suppression, and patients should be warned about infections such as **sore throats** and about the need to **monitoring CBC**.
- **Radioactive iodine** used in resistant or relapsing cases;
- **Surgery** is rarely indicated, if there is a large toxic goiter that is **exerting pressure** or if **drug therapy fails** but *radioactive iodine is contraindicated.*
- **Thyroid function** must be checked regularly, as some patients may become hypothyroid or may relapse after radioiodine or surgery.

Treatment

- The progress of a patient on treatment monitored by estimating plasma **TSH, fT4 and fT3** concentrations, and trying to restore these to normal (although TSH concentration may be slow to normalize).

- **Overtreatment** may induce **hypothyroidism**, with a **rise** in plasma **TSH concentrations** and *low plasma T4/fT4 and T3/fT3*

- In some patients with **severe prolonged hyperthyroidism** a *rise in plasma TSH* may be **delayed** because of the effects of **prolonged feedback suppression of T4** on the pituitary

Subclinical hyperthyroidism

- May occur with a **low or suppressed TSH** but normal (high-normal) **plasma fT4 and fT3 concentrations.**
- May progress to **develope hyperthyroidism** with *suppressed plasma TSH* and *raised plasma fT4 and fT3* concentrations.
- May be associated with **atrial fibrillation**, decreased **bone mineral density** and other features of **hyperthyroidism**.
- **Plasma TSI** may be raised.

Lab investigation

- A careful history (including drugs) should be taken, examination checking for a goiter.
- *Plasma TSH, fT3 and fT4* concentrations should be measured.
- The *plasma fT4 and fT3* concentrations are **clearly high** and the *TSH concentration is suppressed* in clinically thyrotoxic patients.
- **Suppressed plasma TSH**, a clearly elevated plasma fT3 concentration confirms the diagnosis of ***hyperthyroidism***.
- **In T3 thyrotoxicosis** the plasma *fT4 may be normal*.
- *If the plasma fT4* concentration is **raised** and the *TSH* concentration is **normal**, this is suggestive of biochemical **euthyroid hyperthyroxemia** .

Lab investigation

- *Measurement of thyroid antibodies* is useful, particularly if the *concentration of TSIs* is raised which supports a diagnosis of **Graves' disease**.
- The rare **TSH-secreting pituitary tumors** need pituitary assessment and *alpha subunit* concentrations may be useful, as they are usually **raised** in such circumstances.
- **In difficult cases**, determination of plasma *SHBG concentration* as it is lowered in hypothyroidism and raised in hyperthyroidism.
- **Radioiodine uptake** studies of the thyroid can be useful in some of the causes .
- **The TRH test** is sometimes useful in the diagnosis of unclear cases.

Euthyroid goiter

- If **plasma T4 concentrations fall**, enlargement of the thyroid gland (goitre) caused by TSH stimulation.
- **Thyroxine synthesis** may be impaired by **iodide deficiency**, drugs such as **para amino salicylic acid**, or possibly by partial **deficiency of the enzymes** involved in **T4 synthesis**.
- **Prolonged stimulation by TSH the** number of thyroid cells increases and plasma thyroid hormone concentrations are maintained at the expense of the development of a goiter.

Euthyroid goiter

- **Inflammation of the thyroid gland** (thyroiditis),acute or sub acute may produce ***marked but temporary aberrations*** of TFTs.

- **Ultrasound scanning** useful in the diagnosis of goiter as can radiolabelled uptake studies to see if there are *hot T4-producing* or *cold nonproducing* **nodules**.

Sick euthyroid’

- **Any severe illness** may be associated with low plasma total or fT4 concentrations, make the **interpretation of TFTs difficult.**
- **Plasma TSH concentrations** may be normal or slightly high or low.
- The **TSH response to TRH** may also be impaired.
- There may be **impaired conversion of T4 to T3 with low** plasma T3 concentrations.
- the **assessment of thyroid function** is best deferred until the patient has recovered from the illness.

Euthyroid hyperthyroxinemia

- Either the **plasma total or fT4 concentration** is abnormally raised without clinical evidence of thyroid disease.
- Changes may be **transient or persistent**,
- High normal or low total or fT3 concentrations.

Euthyroid hyperthyroxinemia

Causes

- **Physiological conditions** resulting in raised plasma TBG concentration, for example pregnancy.

Concentrations of total T4 and T3 are elevated, but there are usually normal fT4 &fT3 concentrations. **TBG concentration** is raised in newborn babies.

- **Hereditary causes**

– hereditary TBG excess is X-linked,

– hereditary transthyretin excess,

– familial dysalbuminaemic hyperthyroxinaemia (FDH) due to an **abnormal form of albumin**.

Euthyroid hyperthyroxinaemia

Drugs causing hyperthyroxinemia:

– Estrogens, 5-FU, heroin and methadone, raise TBG concentration, resulting in an **elevation of T4 and reverse T3** concentrations,

– Heparin due to **fatty acid release**, *inhibits fT4 binding* to TBG,

– Propranolol inhibits **extra thyroidal conversion of T4 to T3**.

Euthyroid hyperthyroxinaemia

- In some **hyperemesis gravidarum**, have low total and fT3 concentrations due to reduced peripheral conversion of T4 to T3 because 5-deiodinase is inhibited. This results in **elevated** total T4 and fT4 concentrations.

- **Some hepatic disorders**, including acute hepatitis, result in raised concentrations of **TBG and T4 and fT4.**

- In **up to 10%** of cases of acute psychosis, total and fT4 concentrations are raised. It may be due to *central activation of the hypothalamic–pituitary axis*.

Amiodarone and thyroid function

Amiodarone can induce hypothyroidism, partly because it **interrupts the conversion of T4 to T3**.

It contains **iodine** and can also induce thyrotoxicosis by the *Jod–Basedow or type 1 phenomenon*.

- Conversely, it may cause *disruptive thyroiditis* and *thyrotoxicosis* with raised **IL-6 concentration** (type 2 phenomenon).

- The drug has a **long half-life** (40–100 d) and thus takes a long time to clear from the body

Effect of drugs

- Androgens reduce TBG
- Carbamazepine and Phenytoin increased T4 to T3 conversion
- Carbimazole& Propylthiouracil, therapeutic if thyrotoxic
- Lithium may inhibit iodination
- Estrogens increased TBG
- Propranolol blocking T4 to T3 conversion
- Salicylate effect on TBG binding
- Some radio-contrast media blocking T4 to T3 conversion (transient effect)

Case1

57-year-old woman consulted her general practitioner because of weight gain, constipation and weakness. The following thyroid function test results were returned:

Plasma (TSH) 54.6 mU/L (0.20–5.0)

(fT4) 5.7 pmol/L (12–25)

DISCUSSION

- The results show *primary hypothyroidism* with high plasma TSH and low fT4 concentrations. The symptoms are typical of **hypothyroidism**. The patient was also shown to have positive thyroid antibodies (anti-thyroid peroxidase). The thyroid function tests normalized on treatment with **100 µg a day of thyroxine**.

Case2

49-year-old woman was investigated in the medical out-patient department for tiredness. The following test results were returned:

Plasma (TSH) 10.6 mU/L (0.20–5.0)

Free thyroxine (fT4) 13.9 pmol/L (12–25)

DISCUSSION

- These results are suggestive of **compensated hypothyroidism**, in which the *plasma TSH* concentration is *raised* and the *fT4* concentration still remains *within the reference range*.
- The patient also had *positive thyroid antibodies* (anti-thyroid peroxidase). A trial of **thyroxine of 50 µg a day** brought her plasma TSH concentration to within the reference range and improved her symptoms.

Case3

29-year-old woman was seen in the thyroid clinic because of exophthalmos and a goiter. She had the following thyroid function test results:

Plasma (TSH) < 0.05 mU/L (0.20–5.0)
(fT4) 68.8 pmol/L (12–25)
(fT3) 18.7 pmol/L (3–7)

DISCUSSION

The patient had biochemical results typical of **hyperthyroidism**. In fact she had **Graves'** disease and was shown to have positive thyroid-stimulating immunoglobulins and increased diffuse radiolabelled iodine uptake by the thyroid gland.

Compare these results with those of another patient (a 54-year-old woman) in the same clinic:

- **Plasma TSH < 0.05 mU/L (0.20–5.0)**
- **Free T4 18.1 pmol/L (12–25)**
- **Free T3 14.4 pmol/L (3–7)**
- Here, the plasma fT4 concentration is within the reference range, but the plasma fT3 concentration is raised, with suppressed plasma TSH concentration, **suggesting T3 thyrotoxicosis**

Case 4

45-year-old man was on the coronary care unit the day after an acute myocardial infarction. One of his doctors thought that he looked hypothyroid and requested thyroid function tests, the results of which were as follows:

Plasma (TSH) < 0.05 mU/L (0.20–5.0)

(fT4) 10.1 pmol/L (12–25)

(fT3) 1.4 pmol/L (3–7)

On repeating the tests 3 months later at a follow-up appointment in the medical out-patient department, the following results were obtained:

Plasma TSH 2.3 mU/L (0.20–5.0)

Free T4 18.1 pmol/L (12–25)

Free T3 4.5 pmol/L (3–7)

DISCUSSION

The first set of results could indicate **hypothyroidism due to pituitary or hypothalamic defects** (secondary hypothyroidism), that is, low TSH and 'normal' fT4 and fT3 concentrations.

However, the normalization of the results when the patient was not acutely ill suggested **sick euthyroidism or non-thyroidal illness.**

Beware of requesting thyroid function tests in acutely ill patients.

Disorders of haem metabolism

2019

HAEM METABOLISM

- Most body tissues synthesize haem. In bone marrow it is incorporated into haemoglobin, an iron-containing pigment that carries oxygen from the lungs to tissues, and in muscle into myoglobin, which also binds oxygen.
- In other cells it is used for the synthesis of cytochromes and related compounds. The former are components of the electron transport chain, which is involved in oxidative phosphorylation.
- Quantitatively the liver is the major non-erythropoietic haem producing organ. All haem pigments contain iron; the oxygen-carrying ability of the haem molecule depends on the presence of ferrous iron (Fe^{2+}), the form normally present in both haemoglobin and oxyhaemoglobin.

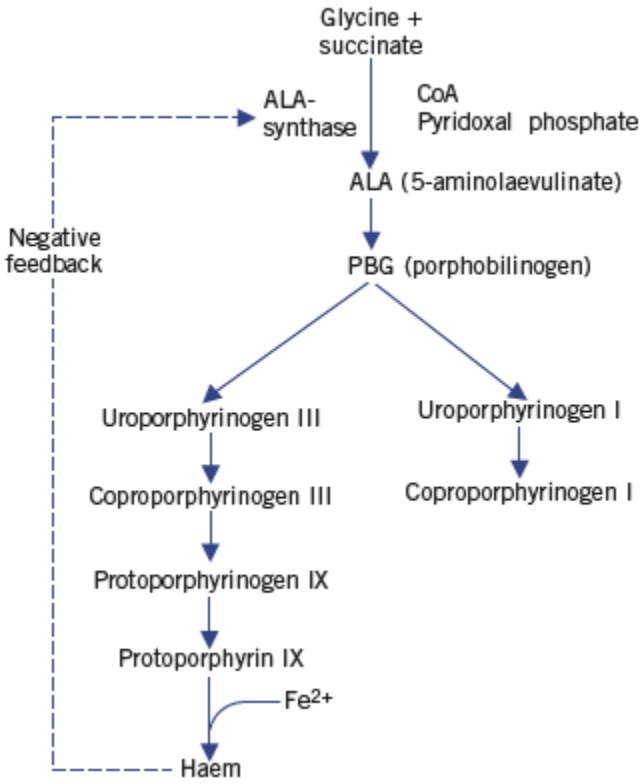
HAEM METABOLISM

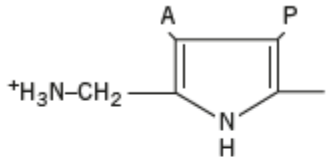
- Red blood cells are broken down by the reticuloendothelial system, predominantly in the spleen. Released haemoglobin is split into the peptide chain, globin, which enters the general protein pool, and haem. The haem ring is split, a process catalysed by haem oxidase, to form a linear molecule, biliverdin. Released iron is reused and biliverdin is reduced to lipid-soluble bilirubin.

Biosynthesis of haem and haemoglobin

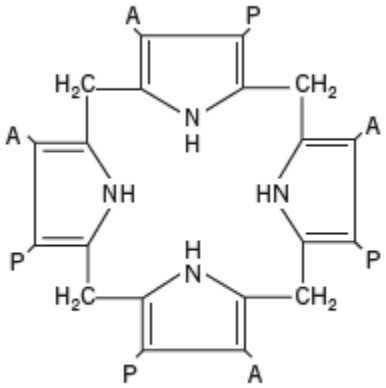
- 5-Aminolaevulinic acid (ALA) is formed by condensation of glycine and succinate. The reaction requires pyridoxal phosphate and is catalysed by ALA synthase. This is the rate-limiting step in the synthetic pathway and is regulated by feedback inhibition by haem.
- Two molecules of ALA condense to form a monopyrrole, porphobilinogen (PBG).
- Four molecules of PBG combine to form a tetrapyrrole ring, uroporphyrinogen . Two isomers are formed, I and III. The major pathway involves the III isomer.
- Haem is formed by the successive production of coproporphyrinogen and protoporphyrin, followed by incorporation of Fe^{2+} into the centre of the ring.
- Haemoglobin consists of four haem molecules, covalently linked to four (two pairs of) polypeptide chains.

Biosynthesis pathways of haem. CoA, coenzyme A.

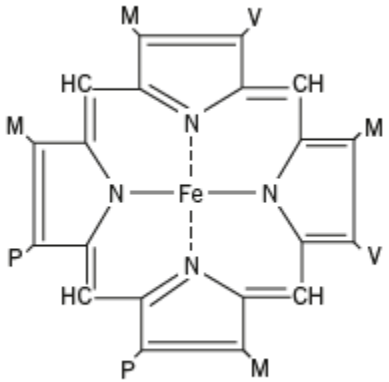




Porphobilinogen



Uroporphyrinogen III



Haem

Excretion of haem precursors

- Excess intermediates on the haem pathway are excreted in either urine or feces.
- The porphyrin precursors ALA, PBG and uroporphyrinogen are water soluble and appear in the urine. They are colourless, but PBG may spontaneously oxidize to form uroporphyrin when exposed to air and light.
- Porphyrinogens also oxidize spontaneously to the corresponding porphyrins, which are dark red and fluoresce in ultraviolet light. A urine specimen containing large amounts of porphyrinogens or their precursors will gradually darken if left standing.
- Protoporphyrin is excreted in bile and appears in the faeces, whereas coproporphyrin(ogen) may be excreted by either route.

Haemoglobin and related compounds

- When oxygen is incorporated into haemoglobin to form oxyhaemoglobin, the spatial arrangement of the haem complexes is altered in such a way as to facilitate further oxygen uptake.
- Other compounds related to haemoglobin may sometimes be formed and some of these may hinder the oxygen-carrying capacity.

Carboxyhaemoglobin

- Carboxyhaemoglobin is cherry red in color and is formed when carbon monoxide binds to haemoglobin or displaces oxygen from oxyhaemoglobin; haemoglobin has a greater affinity for CO than for O2. This occurs in carbon monoxide poisoning, which can be lethal. Once CO is removed from inspired air, oxyhaemoglobin is reformed.
- Consciousness is not lost until the carboxy form has replaced about half the oxyhaemoglobin.

Methemoglobin

- Methemoglobin is hemoglobin in which iron is in the ferric (Fe3+) form (hemin); therefore, it cannot carry oxygen. It is brown and is normally present in very low plasma concentrations; drugs such as sulphonamides or nitrites/nitrates may increase methaemoglobin. The symptoms of methemoglobinaemia are due to hypoxia, which causes cyanosis and an increased respiratory rate and, if methemoglobin is greater than 70% of the total hemoglobin, can be fatal. Methemoglobin may cause a pulse oximeter to read about 85% regardless of the actual amount of oxygen saturation. Methemoglobinaemia is associated with (G6PD) deficiency

Methaemalbumin

- Methaemalbumin is also brown. It is formed when haem combines with plasma albumin in conditions such as severe intravascular haemolysis or acute haemorrhagic pancreatitis when haemoglobin has been converted to haemin in the abdominal cavity and absorbed.
- Methaemalbumin occurs when the haemoglobinbinding capacity of haptoglobin has been exceeded.

Sulphaemoglobin

- Sulphaemoglobin is similar to methaemoglobin but contains sulphur; unlike methaemoglobin, it cannot be reconverted to haemoglobin in vivo. It remains in intact red blood cells. Methaemoglobinaemia-producing drugs cause sulphaemoglobinaemia if hydrogen sulphide is present, usually in the gut.

Myoglobin

- Myoglobin, a haem–protein complex, is normally present in muscle. Plasma concentrations may rise if skeletal muscle, e.g. rhabdomyolysis, or myocardial muscle cells, e.g. myocardial infarction, are damaged.

  Being of low molecular weight, it is rapidly cleared by the kidneys.

IRON METABOLISM

1- Distribution of iron in the body

About 50–70 mmol (3–4 g) of iron are distributed among body compartments. There is considerable interchange of iron between stores and plasma. Free iron is toxic. In normal subjects it is all protein bound: in plasma it is bound to transferrin, in the storage pools to protein in ferritin and haemosiderin, and in erythrocytes it is incorporated into haemoglobin.

About 70 per cent of the total iron is circulating, largely in erythrocyte haemoglobin. However, there are smaller amounts in muscle myoglobin and iron-containing enzymes and cytochromes. Up to 25 per cent of the body iron is stored in the reticuloendothelial system, in the liver, spleen and bone marrow; bone marrow iron is drawn on for haemoglobin synthesis. Iron is stored in cells in the cytosol as ferritin or in the lyosomes as haemosiderin. Ferritin iron is more easily released from protein than that in haemosiderin. Haemosiderin can be seen by light microscopy in unstained tissue preparations.

Ferritin and haemosiderin, but not haem iron, stain with potassium ferrocyanide (Prussian blue reaction), and this staining characteristic may be used to assess

the size of iron stores

Distribution of iron in the body

- Iron deficiency becomes haematologically evident only when no stainable iron is detectable in the reticuloendothelial cells in bone marrow films. Iron overload is likely when, because reticuloendothelial storage capacity is exceeded, stainable iron is demonstrable in parenchymal cells in liver biopsy specimens. This is the storage pool. Only about 50–70 mmol (3–4 mg), or about 0.1 per cent of the total body iron, is circulating in plasma, all bound to transferrin; this fraction is measured in plasma iron assays. This is the transit pool. Iron can cross cell membranes only by active transport in the Fe2+ form; it is in this reduced state in both

oxyhaemoglobin and 'reduced' haemoglobin. It is in the Fe^{3+} form in ferritin and haemosiderin and when bound to transferrin.

Iron absorption

- Body iron content control depends on absorption by an active process in the duodenum: free Fe2+ via the divalent metal transporter 1 (DMT-1) and haem-bound iron via the haem carrier protein 1. Within the enterocyte, some of the iron combines with the protein apoferritin to form ferritin. Free iron is exported from the enterocyte via ferroportin into the circulation where it binds with transferrin for transport and storage. Transferrin-bound iron then enters target cells via receptor-mediated endocytosis. Hepcidin is the main iron regulating protein, and inhibits ferroportin. Concentrations of hepcidin increase in iron overload and reduce in iron deficiency. The human haemochromatosis protein (HFE) regulates the binding of transferrin to the transferrin receptor as well as regulating hepcidin.

Iron absorption

- Normally, about 18 μmol (1 mg) of iron is absorbed each day and this just replaces loss. This amounts to about 10 per cent of the iron ingested in the diet, although the proportion depends to some extent on the type of food.

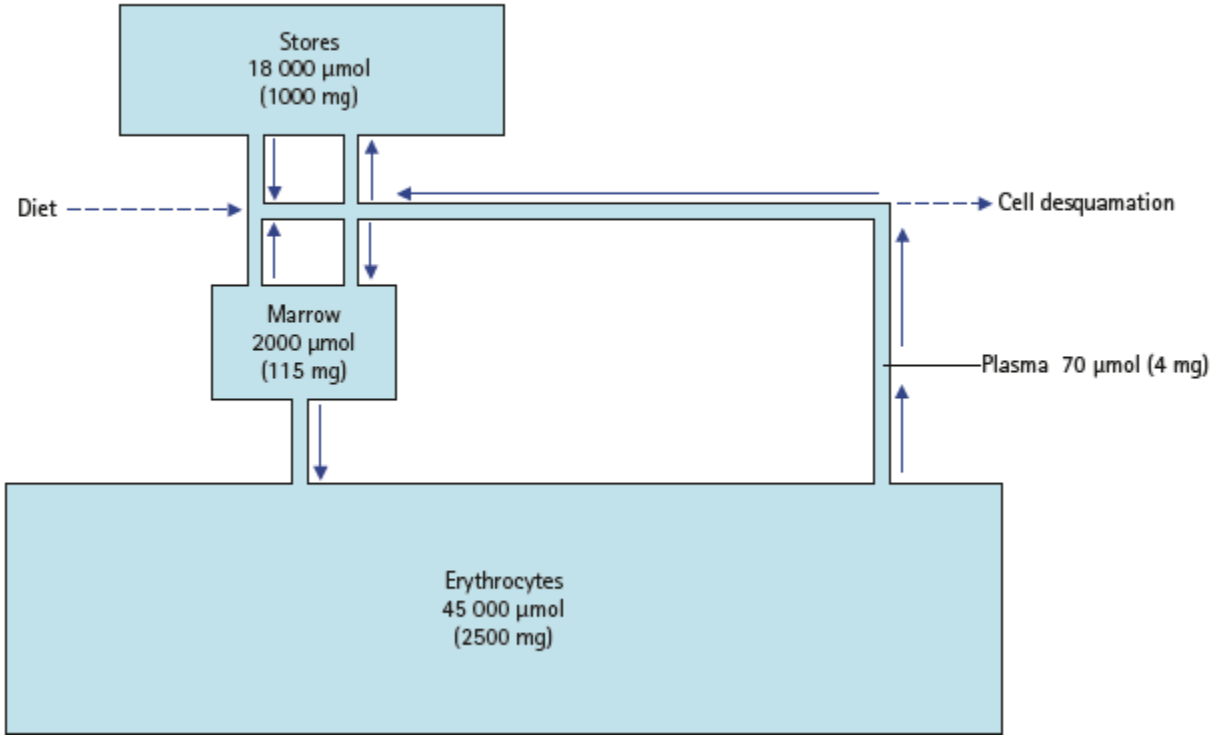
  Iron absorption seems to be influenced by any or all of the following factors:

  oxygen tension in the intestinal cells,
  marrow erythropoietic activity,
  the size of the body iron stores.

  Iron absorption is also increased in many non-iron deficiency anaemias.

  Most normal women taking an adequate diet probably absorb slightly more iron than men and so replace their higher losses in menstrual blood and during pregnancy. Iron requirements for growth during childhood and adolescence are similar to, or slightly higher than, those of menstruating women and can be met by increased absorption from a normal diet.

Body iron compartments.



Iron excretion

- This is poorly controlled, as loss from the body may depend on the ferritin iron content of cells lost by desquamation, mostly into the intestinal tract and from the skin. The total daily loss by these routes is about 18 μmol (1 mg). Urinary excretion is minimal, as circulating iron is protein bound and not water soluble.
- Normal iron loss is so small, and normal iron stores are so large, that it would take about 3 years to become iron deficient on a completely iron-free diet. Of course, this period is much shorter if there is any blood loss, such as menstruation.

Iron transport in plasma

- Iron is transported in the plasma in the ferric form, attached to the specific binding protein transferrin at a concentration of about 18 μmol/L. Transferrin is normally capable of binding about 54 μmol/L of iron and is therefore about one-third saturated. Transferrin bound iron is carried to stores and to bone marrow cells and in the latter some iron passes directly into developing erythrocytes to form haemoglobin.

Factors affecting plasma iron concentration

Sex and age differences

Plasma iron concentrations, like those of haemoglobin and the erythrocyte count, are higher in men than in women, probably for hormonal reasons. The difference is first evident at puberty, before significant menstrual loss has occurred, and disappears at the menopause. Androgens tend to increase the plasma iron concentration and oestrogens to lower it.

Pregnancy and oral contraceptives

In the first few weeks of pregnancy, the plasma iron may rise to concentrations similar to those found in men. A similar rise occurs in women taking some oral contraceptives.

Physiological factors

- Iron concentrations can vary within an individual by up to 100 per cent or more. This can be due to the following:

1- *Random variations Day-to-day variations may be as* much as three-fold and usually overshadow cyclical changes. They may be associated with physical or mental stress or diet, but usually no cause can be found.

2- *Circadian (diurnal) rhythm The plasma iron* concentration is higher in the morning than in the evening. If subjects are kept awake at night, this difference is less marked; it is reversed in night workers.

3- *Monthly variations in women The plasma iron may* reach very low concentrations just before or during the menstrual period. The reduction is probably due to hormonal factors rather than blood loss.

Pathological and clinical factors

- *Any acute or chronic illness (even a bad cold) causing a fall in plasma iron concentration Chronic conditions* such as malignancy, renal disease, rheumatoid arthritis and chronic infections are often associated with normochromic, normocytic anaemia. Iron stores and plasma ferritin concentrations are normal or even increased; the anaemia does not respond to iron therapy. Iron deficiency may be superimposed on the anaemia of chronic illness, especially if drugs are being taken that cause gastrointestinal bleeding; plasma ferritin concentrations are then variable. The finding of hypochromic erythrocytes is the most sensitive index of this complication. Low plasma iron concentrations occur whether or not there is any iron deficiency.
- *Disorders in which the marrow cannot use iron, either* because it is hypoplastic or because some other essential erythropoietic factor, such as vitamin B12 or folate, is deficient; plasma iron concentrations are often high. Blood and marrow films may show a typical picture, but, for example in pyridoxine responsive anaemia and in thalassaemia, the findings in the blood film may resemble those of iron deficiency; in the last two conditions the presence of stainable marrow iron stores excludes the diagnosis of iron deficiency.
- *Haemolytic anaemia The plasma iron concentration* may be high during a haemolytic episode, as iron, liberated from the destroyed erythrocytes, enters the plasma; it is usually normal during the quiescent periods when the iron enters the reticuloendothelial system. Marrow iron stores and plasma ferritin concentrations are usually increased in chronic haemolytic conditions.
- *Acute liver disease Disruption of hepatocytes may* release ferritin iron into the bloodstream and cause a transient rise in the plasma iron concentration. Cirrhosis may be associated with a similar finding, perhaps due to increased iron absorption and intake.

Transferrin and total iron-binding capacity

- Plasma iron concentrations alone rarely give information about the state of iron stores. In rare situations in which doubt remains after haematological investigation, diagnostic precision may sometimes be improved by measuring both the plasma transferrin and the iron concentrations.
- Plasma transferrin can be directly assayed or measured indirectly by adding an excess of inorganic iron to the plasma, any not bound to protein being removed, usually with an exchange resin. The concentration of iron remaining is assayed and the result expressed as the total iron-binding capacity (TIBC).
- Unsaturated iron-binding capacity (UIBC) is the TIBC minus plasma iron concentration. This is usually a valid approximation of the transferrin concentration.
- In rare circumstances, of which the most common is severe liver disease, plasma ferritin concentrations are high enough to bind signifi cant amounts of iron, and the results of iron-binding capacity measurements are then misleading as an assessment of the transferrin concentration.

Physiological changes in the plasma transferrin concentration

The plasma transferrin concentration is less labile than that of iron. However, it rises:

1- after about the 28th week of pregnancy even if iron stores are normal,

2- in women taking some oral contraceptive preparations,

3- in any patient treated with estrogens.

Pathological changes in the plasma transferrin concentration

Plasma transferrin concentration and TIBC:

- rise in iron deficiency and fall in iron overload,
- fall in those chronic illnesses associated with low plasma iron concentrations,
- may be unchanged in acute illness,
- may be very low in the nephrotic syndrome, associated with a low plasma iron concentration, because the relatively low-molecular-weight transferrin is lost in the urine together with iron.

Thus the low plasma iron concentration of uncomplicated iron deficiency is associated with a high transferrin concentration and TIBC; that of non iron deficiency is associated with low concentrations although plasma ferritin is the preferred investigation for iron defi ciency. If iron defi ciency coexists with the anaemia of chronic illness, the opposing effects of the two conditions on the transferrin concentration make it difficult to interpret transferrin, as well as plasma iron, concentrations.

FERRITIN

- Circulating ferritin is usually in equilibrium with that in stores. However, it is an ‘acute-phase’ protein and its synthesis is increased in many inflammatory conditions. The normal plasma ferritin concentration is about 100 μg/L. A plasma ferritin concentration below about 10 μg/L suggests iron deficiency. Results can be misleading if there is coexistent inflammatory disease as accelerated synthesis may lead to normal or even high plasma concentrations despite very low iron stores. In this situation, the results of plasma iron and transferrin assays are also difficult to interpret; haematological parameters remain the most reliable diagnostic indicators of iron deficiency, and possibly also assay of the soluble transferrin receptor.

High concentrations of plasma ferritin usually occur in significant iron overload, but may also be due to:

- inflammatory conditions,
- malignant disease,
- liver disease,
- haemolysis,
- high alcohol intake,
- Still’s disease,
- hereditary hyperferritinaemia.

Thus the finding of a normal or low plasma ferritin concentration almost certainly excludes the diagnosis of iron overload, but a high one does not necessarily confirm it.

	Plasma concentrations				Marrow iron stores
	Iron	Transferrin	Ferritin	TfR	
Low iron concentration					
Iron deficiency	↓	↑	Usually ↓	↑	↓
Acute illness	↓	–	– or ↑	–	–
Chronic illness	↓	↓	– or ↑	–	Usually ↑
High iron concentration					
Early pregnancy	↑	–	–	–	–
Late pregnancy or oral contraceptives	Variable	↑	–	↑	–
Iron overload	↑	↓	↑	↓	↑
Liver disease	↑	↓	↑	– or ↑	May be ↑
Haemolysis	↑	– or ↓	↑	↑	↑

TfR, transferrin receptor; ↑, up; –, normal; ↓, down.

THE SOLUBLE TRANSFERRIN RECEPTOR

- Cells express transferrin receptors (TfRs) on their surface. Plasma or so-called soluble transferrin binds to TfR, preferring the diferric transferrin form.
- The TfR–transferrin complex is internalized into endosomes, where the iron is released into the cytosol.
- The transferrin bound to the TfR returns to the cell surface, where the apotransferrin dissociates and another diferric transferrin can bind. It can therefore be seen that iron uptake into cells is dependent on the number of cell surface TfRs and the concentration and percentage saturation of transferrin.
- Plasma TfR concentrations refl ect total cellular TfR levels, which are increased in iron deficiency as TfR is up-regulated due to the cell's requirements for iron, but, unlike ferritin, are not increased as a result of the acute-phase response of inflammation. Plasma TfR concentrations are also directly related to the rate of erythropoiesis and to increased erythrocyte turnover, such as occurs in haemolytic anaemia.

IRON THERAPY

As the body iron content is determined by control of absorption, rather than excretion, parenteral iron therapy (which bypasses absorption) and repeated transfusions of blood [which contains about 4.5 mmol (250 mg) of iron per unit] may cause iron overload. In anemias other than those due to iron deficiency, stores are normal or even increased.

Proven iron deficiency usually requires iron therapy and the oral route is preferable, as anaphylaxis may occur with parenteral administration. Repeated blood transfusions may be needed to correct severe non iron deficiency anemia, for example if the marrow is hypoplastic, but the danger of overload should be remembered.

Iron absorption is stimulated by anemia even if iron stores are increased. The treatment of non-iron deficiency anemia with oral iron supplements is not only ineffective, but can also lead to iron overload; this is especially likely in hemolytic anemia, in which the iron released from destroyed erythrocytes remains in the body.

IRON OVERLOAD

The only route of iron loss is cell desquamation. Iron absorbed from the gastrointestinal tract or administered parenterally in excess of daily loss accumulates in body stores. If such a 'positive balance' is maintained over long periods, iron stores may exceed 350 mmol (20 g), that is, about five times the normal amount.

Causes of iron overload

- *Increased intestinal absorption:*

– hereditary or primary haemochromatosis,

– anaemia with increased, but ineffective erythropoiesis, for example sideroblastic anaemia, thalassaemia, myelodysplasia,

– liver disease (rare cause),

– dietary excess or iron poisoning,

– inappropriate oral iron therapy.

- *Parenteral administration:*

– multiple blood transfusions,

– inappropriate parenteral iron therapy.

- A very rare cause of iron overload is an inherited deficiency of transferrin.

- Consequences of iron overload
- The effect of the accumulated iron depends on its
- distribution in the body. This, in turn, is influenced
- partly by the route of entry. Two main patterns are seen
- at postmortem or in biopsy specimens.
- *Parenchymal iron overload occurs in*
- haemochromatosis and in patients with ineffective
- erythropoiesis. Iron accumulates in the parenchymal
- cells of the liver, pancreas, heart and other organs.
- There is usually associated functional disturbance or
- tissue damage.
- *Reticuloendothelial iron overload is seen after*
- excessive parenteral administration of iron or multiple
- blood transfusions. The iron accumulates initially in
- the reticuloendothelial cells of the liver, spleen and
- bone marrow.
- In dietary iron overload, both hepatic
- reticuloendothelial and parenchymal overload may
- occur, associated with scurvy and osteoporosis.
- Whatever the cause of massive iron overload, there may
- be parenchymal accumulation and tissue damage.
- *Haemosiderosis is a histological defi nition. An*
- increase in iron stores as haemosiderin can be seen. It
- does not necessarily mean that there is an increase in
- total body iron; for example, in many types of anaemia
- there is reduced haemoglobin iron but increased storage
- iron. *Haemochromatosis describes the clinical disorder*
- due to parenchymal iron-induced damage.

- Syndromes of iron overload
- Hereditary or primary haemochromatosis
- Hereditary haemochromatosis usually has an autosomal recessive mode of inheritance and a defect of the haemochromatosis gene (*HFE). Approximately* 1 in 10 people in Western societies are carriers and about 1 in 1000 are homozygous. The gene for this disorder is closely associated with the human leucocyte antigen (HLA) gene locus on chromosome 6. Some forms of haemochromatosis result in low hepcidin concentrations. Factors such as alcohol abuse may hasten the accumulation of iron and the development of liver damage. Males are more likely to manifest the condition, as menstruation in females lowers tissue iron stores. There is increased intestinal absorption of iron over many years, which produces large iron stores in the tissues, such as the liver, pancreas, joints, heart and gonads. It presents, usually in middle age, as cirrhosis of the liver, sometimes with diabetes mellitus, joint pains, cardiomyopathy, hypogonadism and greyish skin pigmentation due to melanin, not iron. It has been referred to as ‘bronzed diabetes’, although such skin features tend to occur late.

- Examination of liver biopsy specimens may be useful,
- giving an idea of disease severity and showing increased
- iron content when stained by Perl's blue staining,
- although magnetic resonance imaging (MRI) is also
- able to determine iron content non-invasively. There
- is an association with hepatocellular carcinoma, which
- can be screened for by assay of plasma a-fetoprotein
- (AFP).
- The diagnosis may be made on the basis of the
- measurement of plasma ferritin, which is often greater
- than 1000 µg/L. Also plasma iron concentration
- and TIBC, with a saturation of greater than about
- 45 per cent, are indicative of haemochromatosis and
- may increase before plasma ferritin concentration does. The relatives of patients with proven hereditary
- haemochromatosis should be investigated. Genetic
- tests are now available, with a majority of cases of
- haemochromatosis usually being due to the C282Y
- or H63D *HFE gene mutations. There are rarer non-*
- *HFE forms of haemochromatosis associated with gene*
- defects of hepcidin, ferroportin, the transferrin receptor
- and DMT-1.
- Treatment is often by venesection, aiming to reduce
- the plasma ferritin to less than 100 µg/L. Each 500 mL
- unit of blood removes about 250 mg of iron from the
- body.

- Secondary iron overload
- *Anaemia and iron overload*
- Several types of anaemia may be associated with iron overload. In some, such as hypoplastic anaemia and the anaemia of chronic kidney disease, the cause is multiple blood transfusions; the iron initially accumulates in the reticuloendothelial system. If overload is massive (often over 100 units of blood), deposition may occur in parenchymal cells, with the development of secondary haemochromatosis.
- In anaemias characterized by erythroid marrow hyperplasia but with ineffective erythropoiesis, such as thalassaemia major and sideroblastic anaemia, there is, in addition, increased absorption of iron. Secondary haemochromatosis develops at a lower transfusion load than in hypoplastic anaemia.

- Treatment of iron overload of anaemia
- This can obviously not be treated by venesection. The
- tendency for transfusion to aggravate iron overload
- further can be minimized by giving the iron-chelating
- agent desferrioxamine each time; this can be excreted in
- the urine with any non-haemoglobin iron.

- *Dietary iron overload*
- Increased iron absorption due to excessive intake is rare.
- One well-described form is, however, relatively common
- in the rural black population of southern Africa. The
- source is beer brewed in iron containers. Usually, the
- excess is confined to the reticuloendothelial system and
- the liver (both portal tracts and parenchymal cells), and
- there is no tissue damage. In a small number of cases,
- deposition in the parenchymal cells of other organs
- occurs and the clinical picture may resemble that of
- primary haemochromatosis; it may be distinguished by
- the high concentration of iron in the reticuloendothelial
- system seen in the bone marrow. Scurvy and osteoporosis may occur in this form
- of iron overload. The ascorbate defi ciency may be
- due to its irreversible oxidation in the presence of
- excessive amounts of iron, and osteoporosis sometimes
- accompanies scurvy. Ascorbate defi ciency also
- interferes with normal mobilization of iron from the
- reticuloendothelial cells; plasma iron concentrations
- may be low and the response to chelating agents poor,
- despite iron overload.

- Investigation of disorders of iron
- metabolism
- Investigation of iron defi ciency of anaemia
- There are many causes of anaemia, which may be
- due either to iron deficiency or to a variety of other
- conditions, sometimes associated with high iron
- stores. The subject of the diagnosis of anaemia, such
- as haemolytic or pernicious anaemia, is covered more
- fully in textbooks of haematology. Blood haemoglobin
- reflects the major metabolic pool of iron, ferritin the
- storage pool and transferrin the transit pool. The
- clinical impression of anaemia should be confirmed
- by blood haemoglobin estimation. Iron deficiency can,
- however, exist with haemoglobin concentrations within
- the reference range.
- The mean corpuscular volume (MCV) and mean
- corpuscular haemoglobin (MCH) should be checked
- and often a blood fi lm examined. Iron defi ciency
- anaemia is microcytic and hypochromic in type, and
- these fi ndings may be evident before the haemoglobin
- concentration has fallen below the reference range.
- Normochromic, normocytic anaemia is a non-specifi c
- fi nding, usually associated with other chronic disease; it
- is associated with iron defi ciency only if there has been
- very recent blood loss. In most cases of anaemia, consideration of these
- fi ndings together with the clinical picture may elucidate
- the cause. Anaemias such as those due to sickle cell
- disease, thalassaemia or of the sideroblastic type,
- although rarer, are most likely to confuse the picture,
- since they too are hypochromic but are not due to iron
- defi ciency. A low plasma ferritin concentration (less
- than 10 µg/L) is indicative of iron defi ciency due to low
- iron stores.
- One ma[illegible] diagnostic dilemma is to separate iron
- defi ciency anaem[illegible]m anaemia of chronic disorders,
- for example carcinoma or in[illegible]atory disease. In
- the latter there is often an acute-pha[illegible]e, which
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- fi ndings together with the clinical picture may elucidate
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- since they too are hypochromic but are not due to iron
- defi ciency. A low plasma ferritin concentration (less
- than 10 μg/L) is indicative of iron defi ciency due to low
- iron stores.
- One major diagnostic dilemma is to separate iron
- defi ciency anaemia from anaemia of chronic disorders,
- for example carcinoma or infl ammatory disease. In
- the latter there is often an acute-phase response, which
- can raise plasma ferritin concentrations, which are
- infl uenced by infl ammation. This can, therefore, ‘mask’ a

- low plasma ferritin concentration due to iron defi ciency.
- A plasma ferritin concentration more than 200 µg/L
- under these circumstances probably rules out iron
- defi ciency anaemia, but values more than 10 µg/L but
- less than 200 µg/L are equivocal and do not distinguish
- between iron defi ciency anaemia and anaemia of
- chronic disorders. Under these circumstances, plasmasoluble
- TfR assay may be useful; this should be raised in
- iron defi ciency but not in anaemia of chronic disorders.
- An unequivocally low plasma ferritin concentration
- confi rms iron defi ciency, but a normal or high one
- should not be assumed to exclude it. A bone marrow
- fi lm for iron staining may be needed to confi rm the
- diagnosis of iron defi ciency in ambiguous cases. If a
- diagnosis of iron defi ciency is made, it is essential to rule
- out blood loss and its source. Particularly important
- is to exclude gastrointestinal malignancy and also
- gynaecological pathology. Faecal occult blood samples
- may show the presence of gastrointestinal blood loss,
- which may necessitate prompt endoscopic investigation
- (see Chapter 16). A patient with proven iron defi ciency
- who shows no response to oral iron treatment within
- a few weeks may not be taking the tablets. If iron has
- been taken, malabsorption (usually as part of a general
- absorption defect) is a possible explanation of a poor
- response.

- Investigation of suspected iron overload
- Initial tests
- *Plasma iron, percentage saturation of transferrin, plasma*
- *ferritin*
- All of these should be measured. The plasma iron
- concentration is almost invariably high in primary
- haemochromatosis, often more than 36 μmol/L.
- This is associated with a reduced plasma transferrin
- concentration and TIBC, and the percentage
- saturation is usually greater than about 45 per cent,
- and may be nearer 90–100 per cent. In the presence
- of infection or malignancy, however, the plasma iron
- concentration and percentage saturation may be lower
- than expected; the plasma transferrin concentration
- remains low.
- Plasma ferritin concentrations are high in
- most patients with iron overload (whether
- reticuloendothelial or parenchymal). It is rare in
- hereditary haemochromatosis to have a normal plasma
- ferritin concentration, although this may occur in the
- early stages, when the iron saturation may be more
- useful diagnostically. Remember that plasma ferritin
- can be elevated in hepatic disease and high alcohol intake. There is also a rare familial hyperferritinaemia
- due to a defect in ferritin synthesis that should not be
- misdiagnosed as haemochromatosis.
- If all these values are normal, it is unlikely that the
- patient has iron overload. If there is doubt, particularly
- in the face of a positive family history, genetic studies of
- the *HFE gene may be indicated.*

- Demonstration of increased iron stores
- The diagnosis of iron overload usually needs
- demonstration of increased iron stores.
- *Liver biopsy*
- Liver biopsy specimens contain large amounts of
- stainable iron, which may be mainly in parenchymal or
- mainly in reticuloendothelial cells. If haemochromatosis
- is confirmed, liver function tests are necessary, as isexclusion of diabetes mellitus or impaired glucose
- regulation and other clinical features of iron overload
- such as pituitary or gonadal involvement. Cirrhosis may
- progress to hepatocellular carcinoma, which should be
- looked out for.
- *Bone marrow iron content*
- This is usually normal in haemochromatosis, in which
- overload is predominantly parenchymal, but may
- be greatly increased in reticuloendothelial overload.
- A similar loading of the reticuloendothelial cells is
- found when there is deficient use of marrow iron for
- haemoglobin synthesis, as in many haematological,
- neoplastic and chronic inflammatory diseases.
- Family studies
- The blood relatives of patients found to have
- haemochromatosis should be investigated. Estimations
- of plasma iron and percentage saturation of transferrin
- are the most sensitive tests and may change before
- ferritin levels rise if iron stores are not significantly
- increased. Genetic studies looking at *HFE gene*
- mutations are now being used.

- **DISORDERS OF HAEM SYNTHESIS: THE**
- **PORPHYRIAS**
- The porphyrias are a rare group of disorders of haem
- synthesis, resulting from a deficiency of one of the
- enzymes on the haem synthetic pathway (Fig. 21.4).
- Haem production is impaired, but reduced feedback
- inhibition of ALA synthase (the rate-limiting step)
- may maintain adequate haem levels at the expense of
- overproduction of porphyrins or their precursors.
- Porphyrias can be classifi ed into those that
- present with acute porphyric attacks and those withdermatological lesions (cutaneous) but not acute
- porphyric attacks.
- The main porphyrias are as follows:
- The *acute porphyrias:*
- – PBG synthase defi ciency,
- – acute intermittent porphyria,
- – hereditary coproporphyria,
- – variegate porphyria.
- The *cutaneous non-acute porphyrias:*
- – porphyria cutanea tarda, which can be either
- genetic or acquired,
- – congenital erythropoietic porphyria,
- – protoporphyria.
- The last two are also called the erythropoietic
- porphyrias.
- The major clinical and biochemical features are
- outlined in Table 21.2 and discussed briefl y below and
- correlate well with the biochemical abnormalities.

- The acute porphyrias
- Porphobilinogen synthase deficiency is an autosomal
- recessive condition; PBG concentration is not raised,
- although that of ALA is, and the condition is very
- rare. The other three acute porphyrias listed above
- are *autosomal dominantly inherited disorders and*
- have latent and acute phases. The symptoms and
- biochemical abnormalities of the latent phases differ
- and reflect the nature of the enzyme defect. In the acute
- phases, however, the biochemical and clinical pictures
- characteristic of excessive ALA and PBG production
- associated with neurological and abdominal symptoms
- develop. The similarities and differences are best
- explained by referring to a simplified scheme of the
- haem synthetic pathway (see Fig. 21.1).

- Latent phase
- The enzyme defect tends to reduce haem levels, which
- in turn increase ALA synthase activity by a decrease
- in negative feedback inhibition. Increased ALA
- synthase activity has been demonstrated in all the
- porphyrias. Haem levels are maintained at the expense
- of the accumulation and excretion of the substance
- immediately before the block.
- In *acute intermittent porphyria there is increased*
- urinary ALA and PBG excretion, although it may not
- be detectable in all patients; the latent phase is usually
- asymptomatic. It is due to PBG deaminase defi ciency.
- In *hereditary coproporphyria there is increased*
- faecal coproporphyrin excretion; the increase in the
- concentration of porphyrins may produce skin lesions,
- but less commonly than in variegate porphyria. It is due
- to coproporphyrinogen oxidase defi ciency.
- In *variegate porphyria there is increased faecal*
- protoporphyrin excretion; the increase in the
- concentration of porphyrins may produce skin
- lesions. There is no increase in erythrocyte porphyrins;
- however, plasma emission fl uorescence shows a peak
- of 624–626 nm when stimulated at 405 nm, while the
- other acute porphyrias show peaks at 615 nm. It is due
- to protoporphyrinogen oxidase defi ciency.

- *Diagnosis of latent porphyria*
- It is essential to investigate the close blood relatives of
- any patient with porphyria. Screening tests for excess
- urinary PBG and ALA are inadequate to diagnose latent
- acute intermittent porphyria, and even quantitative
- estimation may fail to detect all carriers. The activity of
- the relevant enzymes can be measured in erythrocytes,
- and genetic tests may be useful.

- Acute phase
- Acute disturbances such as peripheral neuropathy or abdominal pain may occur in an acute attack, in which the precursors ALA and PBG are produced in excess. These features may be due to a direct toxic effect of ALA or PBG. Convulsions may occur in acute attacks, sometimes associated with hyponatraemia; this may be due to the syndrome of inappropriate antidiuretic hormone (see Chapter 2).
- 5-Aminolaevulinate synthase activity may be further increased by a number of drugs, particularly barbiturates, estrogens, sulphonamides, phenytoin, halothane and griseofulvin. So many drugs have been implicated that it is probably safer to prescribe from a 'White List' rather than excluding particular drugs. Also important are alcohol and smoking and acute illness such as infection (this may be a direct effect or may be due to an increased demand for haem).
- Acute attacks occur only in a small proportion of patients exposed to the provoking agents. They are more common in premenstrual or pregnant women, and in women rather than in men, and usually occur only after puberty. Colicky abdominal pain, due to involvement of the autonomic nervous system, and neurological symptoms ranging from peripheral neuropathy to quadriplegia are usually the presenting features. Death may result from respiratory paralysis or blood pressure instability due to autonomic neuropathy.

- The acute attack closely resembles serious acute
- intra-abdominal conditions and, if the diagnosis is not
- made, the patient may be subjected to surgery with the
- use of a barbiturate anaesthetic; this and the stress of
- operation may aggravate the condition.
- The acute attack is marked by an increase in ALA and
- PBG production. In acute intermittent porphyria, this
- increase is due to the block imposed by an inherited
- defi ciency of PBG deaminase; in hepatic coproporphyria
- and variegate porphyria, this enzyme becomes rate
- limiting and is unable to respond normally to theincreased demand. *The increase in urinary ALA and*
- *PBG concentrations is the hallmark of the acute porphyric*
- *attack and, in hereditary coproporphyria and variegate*
- porphyria, is superimposed on all the other biochemical
- abnormalities. The accelerated activity of the pathway
- and the spontaneous conversion of the precursors
- to porphyrin lead to increased urinary porphyrin
- excretion. About 1 per cent of acute attacks are fatal. It
- is important to stop precipitating drugs and not to use
- medications that could evoke an attack. Convulsions
- may be treated with gabapentin or vigabatrin. Haem
- arginate, given by slow intravenous infusion, can result
- in a reduction in ALA and PBG concentrations and
- thus reduce some of the features of the acute attack, but
- not usually the neuropathy

- Cutaneous porphyrias (non-acute)
- The cutaneous porphyrias include porphyria cutanea
- tarda, congenital erythropoietic porphyria and
- protoporphyria

- Porphyria cutanea tarda
- This is the most common porphyria. In patients with
- porphyria cutanea tarda, the skin is unduly sensitive to
- minor trauma, particularly in sun-exposed areas; the
- most common presenting feature is blistering on the
- backs of the hands. Less commonly, the lesions appear
- on the face. Increased facial hair and hyperpigmentation
- occur in chronic cases. Acute attacks do not occur, but
- this type of porphyria is associated with hepatic damage.
- The basic defect is an inability to convert
- uroporphyrinogen to coproporphyrinogen due to a
- defi ciency of uroporphyrinogen decarboxylase. There
- are two types of porphyria cutanea tarda:
- It may be an *autosomal dominantly inherited disorder.*
- The familial form is rare, although environmental
- factors are also important.
- Most cases are acquired. Factors that produce clinical
- disease, possibly by aggravating an underlying geneticdeficiency, include alcohol abuse, iron overload or
- high-dose estrogen therapy. Symptoms improve when
- the offending substance is withdrawn. Some liver
- toxins such as hexachlorobenzene directly inhibit the
- activity of the enzyme. There is an association with
- hepatitis C. Iron overload can also be present and
- haemochromatosis needs to be excluded.
- The impaired conversion leads to an accumulation
- of uroporphyrinogen and porphyrins intermediate
- between it and coproporphyrinogen. These deposit
- in the skin and are excreted in the urine in increased
- amounts. Faecal porphyrins are not increased but the
- abnormal pattern of intermediate porphyrins may
- be detected by chromatography; this fi nding is of
- diagnostic value. It is important not to confuse this
- disorder with the coproporphyrinuria of liver disease
- (see below).

- Erythropoietic porphyrias
- Two rare inherited disorders are associated with the
- accumulation of porphyrins in erythrocytes. Acute
- porphyric attacks do not occur and ALA and PBG
- excretion are normal.
- *Congenital erythropoietic porphyria*
- Congenital erythropoietic porphyria is inherited
- as an *autosomal recessive characteristic due to*
- uroporphyrinogen III synthase deficiency. Usually, blood
- erythrocyte and plasma uroporphyrin I concentrations
- are very high from infancy onwards and there is severe
- photosensitivity. Porphyrins are also deposited in bones
- and teeth, which fluoresce in ultraviolet light; the teeth
- may be brownish-pink in colour. Hirsutism, especially
- of the face, also occurs and there is haemolytic anaemia.
- Urinary porphyrin concentrations are grossly increased,
- although faecal porphyrin levels are less so.

- *Protoporphyria*
- This is an *autosomal dominantly inherited disorder due*
- to ferrochelatase deficiency in which protoporphyrin
- concentrations are increased in erythrocytes and faeces.
- There is mild photosensitivity, and hepatocellular
- damage may lead to liver failure.
- These two conditions tend to give rise to
- photosensitivity, whereas there is increased skin fragility
- in the other porphyrias that involve the skin. The
- erythropoietic porphyrias can be treated by sunlight
- avoidance, for example by using skin sunblocks. In
- porphyria cutanea tarda, venesection may be used to
- reduce iron stores and oral chloroquine to increase theexcretion of porphyrins. Carotene treatment may be
- useful in erythropoietic porphyria

- Other causes of excessive porphyrin
- excretion
- Porphyria is not the only cause of disordered porphyrin
- metabolism, and positive screening tests *must be*
- *confirmed by quantitative analysis, with identification*
- of the porphyrin. Other causes must be considered.
- Lead poisoning
- Lead poisoning inhibits several of the enzymes
- involved in haem synthesis, including PBG synthase
- and ferrochelatase, and eventually causes anaemia. The
- urine contains increased amounts of ALA (an early
- and sensitive test), and coproporphyrin. Some of the
- symptoms of lead poisoning, such as abdominal pain
- and peripheral neuropathy, are similar to those of the
- acute porphyric attack, and may cause difficulty in
- diagnosis. However, the excretion of PBG is not usually
- increased. Zinc protoporphyrin concentration rises
- with increased lead exposure, although the method of
- choice for assessing exposure to inorganic lead is blood
- lead concentration. Other features of lead poisoning
- include lead staining of the teeth and basophilic
- stippling of red blood cells.
- Liver disease
- This may increase urinary coproporphyrin levels,
- possibly because of decreased biliary excretion. It is
- probably the most common cause of porphyrinuria.
- Occasionally there is mild photosensitivity (in
- porphyria cutanea tarda, the more severe skin lesions
- are due to uroporphyrin excess). Bleeding lesions of upper gastrointestinal tract
- These lesions may produce raised levels of faecal
- porphyrin by degradation of haemoglobin. If there is
- bleeding from the lower part of the tract, the blood
- reaches the rectum before there is time for conversion;
- this may be of help in locating the approximate site of
- bleeding.
- Iron deficiency and [illegible]oblastic anaemia
- This may also result in increa[illegible]centrations of
- erythrocyte porphyrins.

- Investigation of suspected porphyria
- The laboratory should be notified and a check made
- of which type of samples is required. Porphyrinsare relatively stable, but samples must be protected
- from light. Porphobilinogen rapidly polymerizes to
- uroporphyrins and consequently a random fresh
- urine sample is more suitable than a 24-h collection.
- All samples should be analysed as soon as possible
- after collection. Samples should not be sent simply
- requesting a 'porphyrin screen'; an indication should
- be given of which type of porphyria is suspected, giving
- the relevant clinical details, so that the laboratory can
- select the appropriate tests.
- Generally, urine, faeces and blood (both plasma and
- erythrocytes) are needed for complete analysis. The
- technique of plasma fl uorescence has recently proved
- useful to distinguish some of the porphyrias (Table
- 21.3). Specialist laboratories can carry out enzyme
- assays and genetic tests.

- Suspected acute porphyria
- Fresh urine should be tested immediately for PBG
- using Ehrlich's reagent. If PBG is not present, it is highly
- unlikely that the patient is suffering from an acute
- porphyric attack unless he or she has the rare PBG
- synthase deficiency, in which case ALA concentration
- would be expected to be raised. A patient with a history
- of repeated attacks of abdominal pain or neurological
- symptoms may have acute intermittent porphyria,
- variegate porphyria or hereditary coproporphyria.
- If an acute porphyria is confi rmed, the concentration
- of porphyrins in urine and in a random sample of faeces
- should be measured. Raised values suggest variegate
- porphyria (protoporphyrin and coproporphyrin) or
- hereditary coproporphyria (coproporphyrin). Acute
- intermittent porphyria does not normally show abnormal faecal porphyrins. The following should be
- remembered.
- Patients with acute intermittent porphyria who
- have once had an acute attack may continue to excrete
- increased amounts of PBG for many months. This
- condition does not show skin lesions, unlike the other
- acute porphyrias. Plasma fl uorescence may reveal
- different emission peaks when stimulated at 405 nm,
- for example variegate porphyria may show a peak at
- 625 nm when stimulated at 405 nm, whereas the other
- acute porphyrias show a peak at 615 nm.
- It is possible to assay enzymes of haem metabolism in
- red blood cells. For example, decreased PBG deaminase
- is found in patients with acute intermittent porphyria.
- There is often overlap in levels between affected and
- unaffected people but, within a family, carriers can be
- shown to have levels about half those of unaffected
- members. This test may also detect affected children.
- However, the enzyme activity is related to the mean
- age of the red blood cells; this assay is not suitable
- for children under the age of about 9 months or for
- individuals with haemolytic anaemias. Negative urine
- and faeces porphyrin tests do not exclude the diagnosis
- of latent porphyria, and in these cases enzyme and
- genetic tests may be useful.

- Suspected porphyria with skin lesions
- Skin lesions may occur in any type of porphyria
- other than acute intermittent porphyria. Blood,
- urine and faeces should be sent for testing. Increased
- concentrations of erythrocyte porphyrins suggest
- protoporphyria or congenital erythropoietic porphyria(very rare). High concentrations may also occur in iron
- deficiency anaemia and lead poisoning.
- Increased concentrations of urinary porphyrins
- suggest porphyria cutanea tarda or congenital
- erythropoietic porphyria. The increased uroporphyrin
- excretion in these conditions must be distinguished
- from the coproporphyrinuria of liver disease.
- Increased concentrations of faecal porphyrins occur
- in protoporphyria, variegate porphyria and hereditary
- coproporphyria. These may be distinguished by
- chromatographic separation of porphyrins; this will
- also demonstrate the abnormal pattern of porphyria
- cutanea tarda.
- Protoporphyria shows a unique plasma fl uorescence
- emission peak of 632 nm when stimulated at 405 nm.
- Enzyme and genetic tests may be useful

- Investigation of family members
- If one of the porphyrias is diagnosed, it is essential to
- investigate blood relatives. Those found to have the
- condition must be counselled regarding the medications
- that may precipitate an attack. King George III was
- thought to have an acute porphyria, although it has not
- been proven (Fig. 21.5).
- Carriers of variegate porphyria and hereditary
- coproporphyria may be identifi ed after puberty by the
- demonstration of clearly increased faecal porphyrin
- excretion. Normal excretion before puberty does not
- exclude the diagnosis. Some of the porphyrias may be
- diagnosed by enzyme determination, for example acute
- intermittent porphyria is detected by measuring redcell PBG deaminase activity. Genetic studies are now
- possible in specialist laboratories to investigate the
- porphyrias.

ENZYMES

2019

ENZYMES

- Enzymes are biological catalysts of protein nature.
- No chemical process in the living organisms can be effected without involvement of enzymes.
- At the present time, the ***enzymology*** is an extremely important, rapidly growing branch of biochemistry, and its achievements are widely used in practical medicine, pharmaceutics, food industry, and other related industries.

Common and distinct features in enzymes and non -enzymic catalysts

Enzymes and non- biological catalysts, in obeying the general laws of catalysis, share the following common features:

1. They catalyze dynamically possible reactions only.

2. They never alter the reaction route.

3. They go not affect the equilibrium of a reversible reaction, but rather accelerate its onset.

4. They are never consumed during the reaction. Therefore, a cellular enzyme functions until it becomes impaired for one or another reason.

Enzymes show a number of features that distinguish them from non -biological catalysts.

1. The **rate of enzymic catalysis** is much greater to that of non enzymic catalysis. It follows there from that enzymes lower the activation energy of reactions to a greater extent as compared with non-biological catalysts.

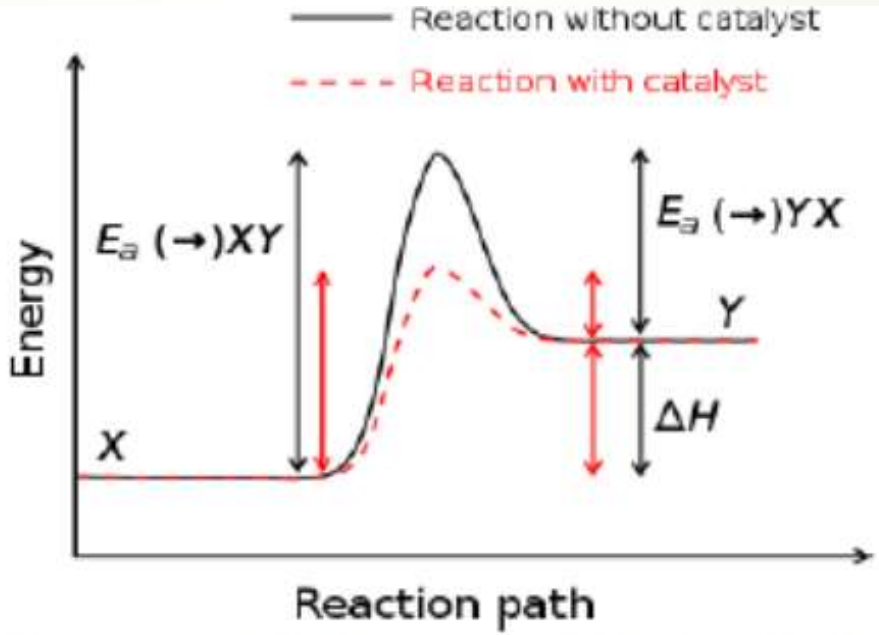
2. Enzymes exhibit a high specificity. There are enzymes that act **selectively** on only one stereo isomer of a compound. The high specificity of enzymes enables them to direct metabolic processes to strictly defined channels.

3. Enzymes catalyze **chemical reactions under "mild' conditions**, i.e. at normal pressure, low temperature (about +37°C), and pH close to that of the neutral medium (pH=7±1). This behavior differentiates them from other catalysts active at high pressure, extreme pH values, and high temperature. Enzymes, because of their proteinic nature, are susceptible to temperature variations (i.e. are thermo-labile) and to the change of medium pH.

4. Enzymes are catalysts with **controllable activity**, the behavior never encountered in non biological catalysts. This unique property in enzymes allows changing the rate of metabolism in the organism depending on the environmental conditions, i.e. adjusting the metabolic activity to the action of various factors.

5. The **rate of an enzymic reaction** is proportional to **the amount of enzyme**, while no strictly defined relationship of this kind is found in non biological catalysts. Therefore, the short supply of an enzyme in the living organism signifies a lower rate of metabolism and, on the contrary, the additional production of an enzyme is one of the adaptive routes for the organism cells.

- The highest energy position (peak position) represents the transition state. With the catalyst, the energy required to enter transition state decreases, thereby decreasing the energy required to initiate the reaction.



The relationship between activation energy (E_a) and enthalpy of formation (ΔH) with and without a catalyst

Classification and nomenclature of enzymes

the cell contains about 104 enzyme molecules capable of catalyzing over 2000 various reactions. About 150 enzymes have been isolated in the crystalline form.

There are 2 classifications of enzymes:

1. The trivial (working) classification

The trivial name for an enzyme was made up of the ending ***–ase*** added to the name of the substrate subject to the action of the enzyme, ***saccharose + ase = saccharase***

2. The systematic classification

The systematic name for an enzyme is constructed in a more complicated manner. It is made up of the names of substrates of the chemical reaction catalyzed by the enzyme, *the name of the type of the catalyzed chemical reactions,* and the ending *–ase*. the systematic name for the enzyme *lactate dehydrogenase* is written as:

L-lactate:	***NAD+***	***-Oxidoreductase***
Substrate- 1	substrate- 2	type of chemical reaction

Classification and nomenclature of enzymes

The systematic names are given to the search enzymes only.

1. oxidoreductases
2. transferases
3. hydrolases
4. lyases
5. isomerases
6. ligases (or synthetases).

Classification and nomenclature of enzymes

The name of the group indicates the type of the chemical reaction catalyzed by enzymes. According to the numerical classification system, each enzyme receives a four-part number whose numerals are separated by a dot:

LACTATE DEHYDROGENASE **E.C. 1. 1. 1. 27**

- *Enzymatic classification* _____↑
- *group number* _____↑
- *sub- group number* _____↑
- *sub- subgroup number* _____↑
- *ordinal number in sub- subgroup* _____↑

All new enzymes are classified only in accordance with the recommendations of the **Committee on Enzyme Nomenclature of the International Union of Biochemistry**

Classification and nomenclature of enzymes

- **Oxidoreductases** are enzymes that catalyze redox reactions. The substrate subject to oxidation with oxidoreductases is regarded as a hydrogen donor. For this reason, the enzymes in this group are called **dehydrogenases,** or, less commonly, **reductases**. They play a significant role in the energy metabolism.
- **Transferases** are enzymes that catalyze reactions of transfer of various moieties from one substrate (donor) to another (acceptor). The enzymes that catalyze the transfer of methyl groups are called *methyl transferases*; those that catalyze the amino group transfer are called *amino transferases*, etc.
- **Hydrolases** are enzymes that catalyze the substrate bond cleavage by adding water. The trivial names for hydrolases are made up by adding the ending *–ase* to the name of substrate. Systematic names must, by convertion, contain the term *hydrolase* (amyl*ase*, *saccharase*).

Classification and nomenclature of enzymes

- **Lyases** are enzymes that catalyze bond-cleaving reactions in a substrate without oxidation or addition of water (*pyruvate decarboxylase*).
- **Isomerases** are enzymes that catalyze structural rearrangements within a single molecule (*triose isomerase*).
- **Ligases *(synthetases)*** are enzymes that catalyze the addition of two molecules using the energy of phosphate bond. ATP or other nucleoside phosphates serve as energy sources in the synthetase-catalyzed reactions (*DNA-ligase*).

Structural and functional organization of enzymes

- The enzymes exhibit all features characteristic of the protein structural organization. They possess four levels of organization: primary, secondary, tertiary, and quaternary. The enzymes with quaternary structure are composed of protomers (subunits) and constitute a greater type. Similarly to other functional proteins, they are classified into simple enzyme proteins and conjugated (or compound) enzyme proteins.
- A conjugated enzyme is composed of a **protein portion**, ***apoenzyme***, and a non protein portion, ***cofactor***. The cofactors in enzymes are metal ions and coenzymes. Taken singly, the apoenzyme and cofactor exhibit a low (if any) activity as catalysts, but united, they make up a molecule of the active enzymes, or ***holoenzyme.***

Functional organization of enzyme

In the three-dimensional structure of a simple, as well as a conjugated, enzyme, there are distinguished a number of regions responsible for certain specific functions. A portion of the enzyme molecule constitutes the *active center (center*), i.e. the site in the enzyme spatial structure where the binding with a *substrate (**S**)* takes place (substrate is a compound that undergoes conversion by the action of enzyme).

Alongside of the active center, an enzyme has a *regulatory*, or *allosteric, center* spatially remote from the active center in the enzyme molecule. From name allosteric center suggests that the molecules bound to this center are structurally dissimilar from the substrate but exert influence on the binding and conversion of substrate at the active center by changing the substrate configuration.

The enzyme molecule can have more than one allosteric center. Compounds capable of binding to an allosteric center are called *allosteric effectors*. They exert influence, through the allosteric center, on the function of the active center in a facilitating or an inhibitory manner. Accordingly, allosteric effectors are referred to as positive (activators) or negative (inhibitors).

Structure of the active center

- There are distinguished, in the active center, a *contact site* (or anchor site) for binding a substrate, and a *catalytic site* at which the conversion of the bound substrate takes place. Usually, the enzyme active center is made up of 12 to 16 amino acid residues of a polypeptide chain; occasionally, their number may be larger.

Functional groups of enzyme active center

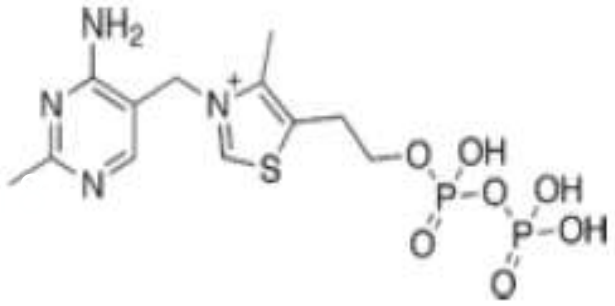
- In simple enzymes, the role of functional groups at the contact and catalytic sites is assigned to the side-chain radicals of amino acids only. In conjugated enzymes, the leading part in these processes is played by cofactors.
- The following functional enzyme groups take part in catalysis: -COOH, -NH2, -OH, -SH and other.

Cofactors and their role in enzyme function

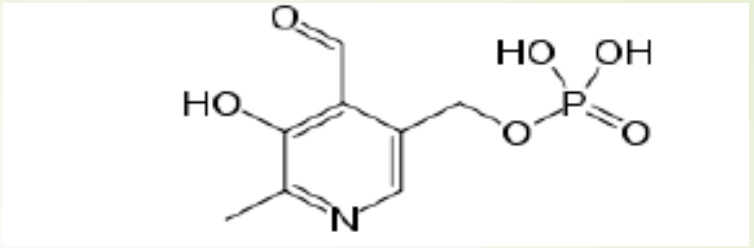
- Cofactors are either bound to the enzyme active center, or susceptible to easy cleavage by dialysis. The term ***prosthetic*** group is applied to tightly-bound cofactors, similar to the accepted definition of the non protein portion in non enzymic protein. Still, such a definition appears to be somewhat arbitrary, since the cofactor (usually coenzyme) can be tightly bound to the active center of one enzyme and none, to that of another enzyme. Vitamins are parent materials for coenzymes, therefore, their dietary deficiency affects the synthesis of these coenzymes and leads, as a consequence, to an impaired function of the corresponding conjugated enzymes.

  Thiamine coenzyme is derived from thiamine (vitamin B1)

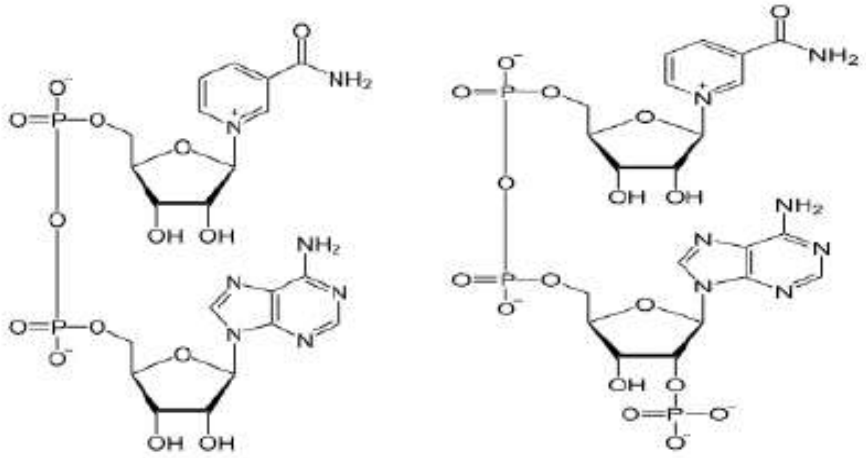
- Niacin (vitamin B5, nicotinic acid) serves as a source for generating nicotinamide coenzymes. The latter species include (NAD) and (NADP). One of the mononucleotides of these coenzymes contains nicotinamide, the other one is represented by adenylic acid.
- ***Pyridoxine coenzyme.*** Pyridoxine coenzyme is derived from pyridoxine (vitamin B6).
- ***Flavin coenzymes.*** These derived from riboflavin (vitamin B2) structurally related to the isoalloxazine derivatives. Coenzymes (FMN) and (FAD) are synthesized from riboflavin.



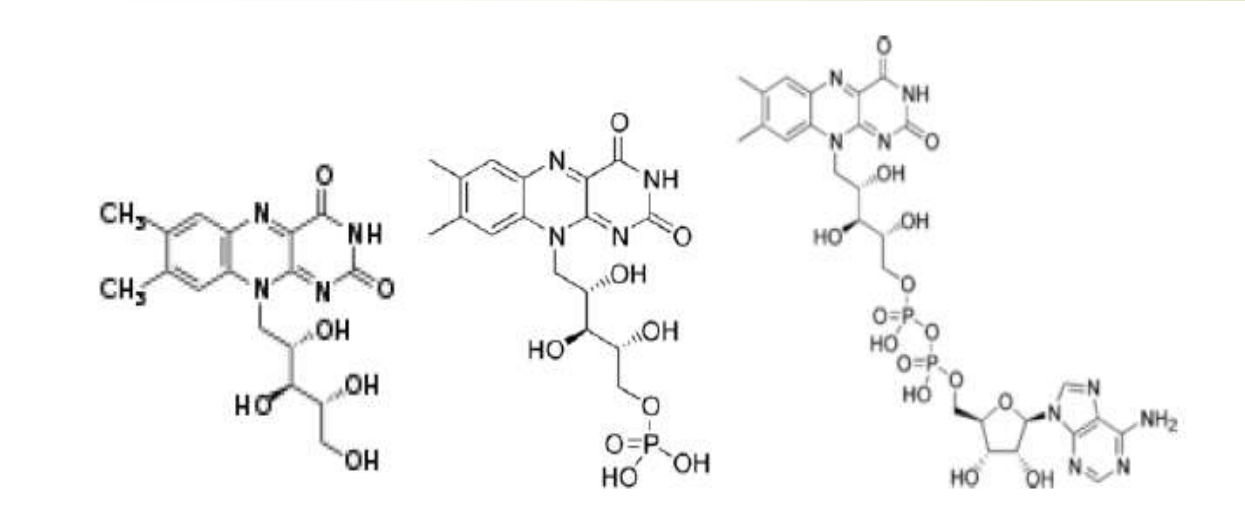
Structure of TPP (or TDP)



Coenzyme of vitamin B6, pyridoxal phosphate



Nicotinamide coenzymes (structures of NAD and NADP)



Chemical structures of vitamin B2 and its coenzymes, FMN and FAD

- ***Metalloporphyrin coenzymes*** include the hemes that participate as coenzymes in redox-reactions catalyzed by oxidoreductases (cytochromes, catalase, peroxidase, etc.).
- ***Metal ions*** are likewise capable of acting as cofactors. Metalloenzymes constitute a very widespread group of enzymes accounting for 25% of all the enzymes. The role of the metal in these enzymes may be quite different. Metalloenzymes are divided into two groups:

1. Enzymes in which the metal ion acts as an activator (these enzymes exhibit catalytic activity in the absence of metal as well).

2. Enzymes in which the metal ion acts as a cofactor (in the absence of metal these enzymes are inactive).

MECHANISM OF ENZYMIC ACTION

The process of enzyme catalysis may conventionally be differentiated into three steps, each exhibiting its specificity.

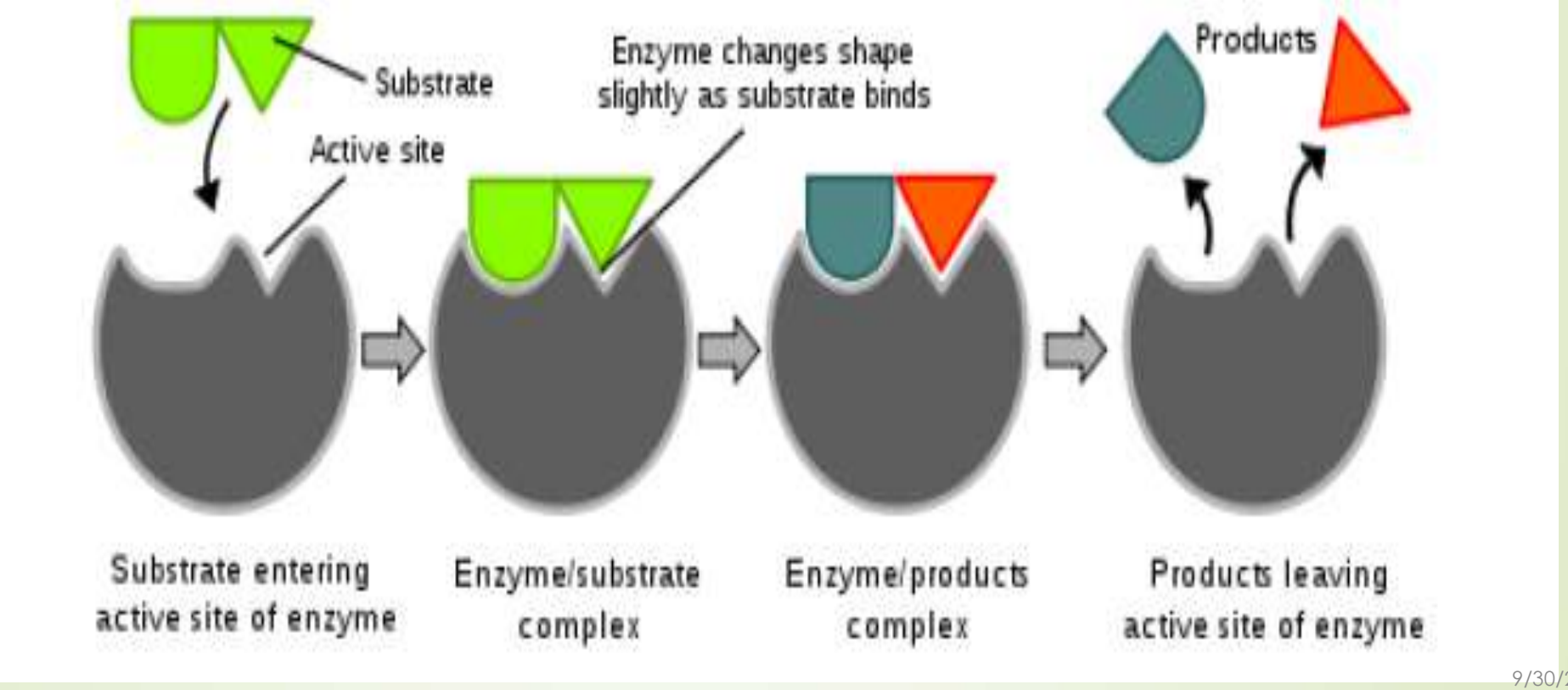
1. Diffusion of a substrate to an enzyme resulting in a stereospecific binding of the former to the active site of enzyme (formation of an enzyme-substrate complex **ES**).

2. Conversion of the primary enzyme-substrate complex into one or more activated enzyme-substrate complexes (denoted **ES*** in the scheme).

3. Detachment of the reaction products from the active center of enzyme and their diffusion into the environment (complex **EP** dissociates into **E** and **P**).

- **$E + S \leftrightarrow ES \rightarrow ES^* \rightarrow EP \rightarrow E + P$**

Diagrams to show the induced fit hypothesis of enzyme action



Specificity of enzyme action

Enzymes show a varying degree of specificity towards substrates..

1. ***Absolute substrate specificity:*** the enzyme catalyzes conversion of a single substrate only. For example, ***urease* is active only in the conversion of urea.**

2. ***Absolute group substrate specificity:*** the enzyme catalyzes conversion of a related group of substrates. For example, ***alcohol dehydrogenase*** catalyzes conversion not only of ethanol, but other alcohols too, though at different rates.

3. ***Relative substrate specificity:*** the enzyme catalyzes conversion of substrates belonging to different groups of chemical compounds. For example, the enzyme ***cytochrome P450*** *(hydroxylase*) participates in the hydroxylation of a large number (about 700) of compounds. Enzymes with relative substrate specificity constitute the least specific enzymic system involved in the conversion of naturally occurring materials, drugs, and toxins.

4. ***Relative group substrate specificity:*** the enzyme exhibits a specific activity towards individual bonds within a group of substrates. For example, **digestive enzymes (*pepsin, trypsin*)** are specific towards the peptide bonds formed between certain amino acid residues in various proteins.

5. ***Stereochemical substrate specificity:*** the enzyme catalyzes conversion of only one among all possible substrate stereoisomers. This is an extreme case of enzymic specificity. For example, ***D-alanine oxidase*** catalyzes the conversion of only *D*-alanine amino acid, and never of its stereoisomer, *L*-alanine amino acid.

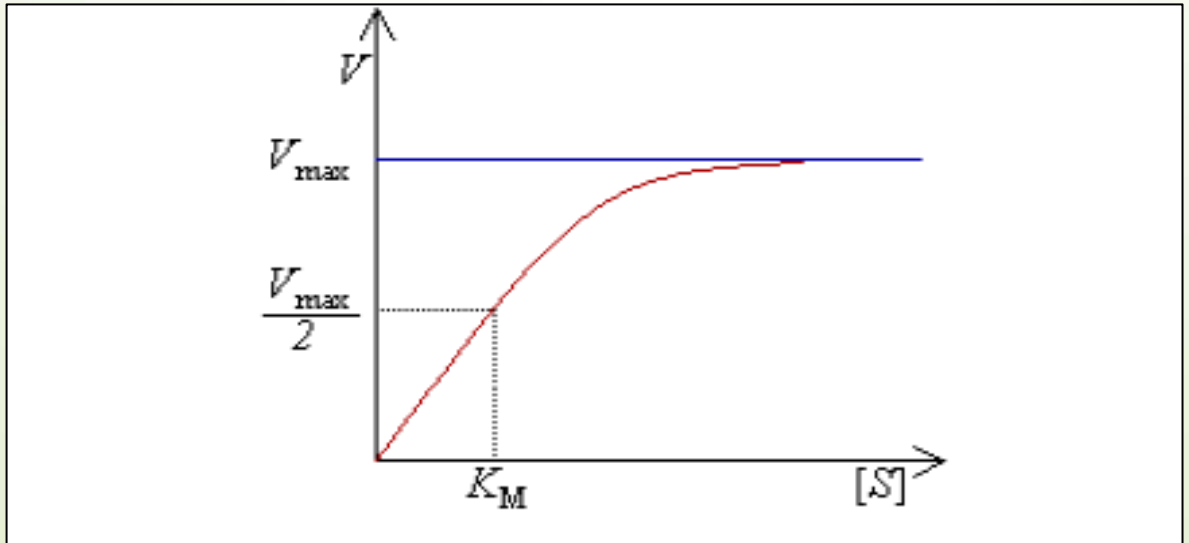
Kinetics of enzymic reactions

- The kinetics of enzymic activity is a branch of enzymology concerned with the studies of enzyme-catalyzed reaction rates as affected by the chemical nature of substrate and enzyme, by the conditions for their interaction, as well as by environmental factors. Otherwise stated, the enzyme kinetics allows one to gain insight into the molecular mechanistic nature of the factors affecting enzymic catalysis rates.
- The **rate of an enzymic reaction** is defined by the amount of a material (or materials) converted per unit time. The rates of such reactions are dependent on the environmental factors (temperature, pH of medium, influence of native or foreign materials, etc.).
- The principles of enzymes' reaction kinetics have been laid down in the works of *Michaelis* and *Menten*. The Michaelis–Menten equation relates the initial reaction rate *v*0 to the substrate concentration [S]. The corresponding graph is a rectangular hyperbolic function; the maximum rate is described as *V* max (asymptote).

$$v_0 = \frac{v_{\max}[S]}{K_M + [S]}$$

Dependence activity of enzyme (V) on concentration of substrate (S)

- The **Michaelis–Menten equation** describes the rates of **irreversible reactions**. A steady state solution for a chemical equilibrium modeled with Michaelis–Menten kinetics can be obtained with the Goldbeter–Koshland equation.
- The rate of an enzymic reaction is seen as a measure for catalytic activity of the enzyme involved and is referred to simply as the activity of enzyme. The enzymic activity can be estimated only in an indirect fashion, by a decrease in the amount of substrate converted, or by an increase in the concentration of products formed per unit time. The dependence of reaction rate on the enzyme amount is linear in character.



Dependence of reaction rate on pH of medium

Usually, the curve relating the enzymic reaction rate to the medium pH has a bell-like shape, since each enzyme is characterized by a pH range within which the rate of an enzyme-catalyzed reaction attains a maximum. Any change in the pH value to either side leads to a decrease in the enzymic reaction rate.

Optimal pH values for certain enzymes:

- pepsin = 1,5 – 2,5
- urease = 6,4 – 7,2
- trypsin = 7,5 – 7,8
- arginase = 9,5 – 9,9.

Dependence of enzymic reaction rate on temperature

- When the temperature of medium is raised, the rate of an enzymic reaction increases, attains a maximum at an optimal temperature, and then drops down to zero. The optimal temperature for most enzymes falls within the range of 20-40°C.
- Thermal liability of enzymes is related to the proteinic structure.
- Certain enzymes are denatured at temperatures above 40-50°C.
- A number of enzymes are inactivated on cooling, i.e. their denaturation occurs at temperatures close to 0°C.

Estimation of enzymic activity: methods and units

- The enzymes contained in cells, tissues, and organs must be preparative extracted by making use of special techniques. The enzyme solution (extract from biological materials) is then used for enzyme testing. Serum or blood plasma, as well as other biological fluids are ready enzyme solutions and can be used for testing immediately. Enzyme tests, both qualitative and quantitative, are carried out in an indirect manner by measuring a decrease in the substrate amount, or by estimating reaction products accumulated in the medium.
- The rate at which the substrate is consumed, or the reaction products are accumulated per unit time, is taken as a measure of *enzymic activity*.
- The determination is carried out using any suitable method (colorimetry, spectrophotometry, fluorometry, polarography, etc.) either by recording the signal after the reaction is interrupted in a certain period of time, or by taking measurements continually during the reaction. The latter technique is more convenient, especially if the substrate or reaction products exhibit characteristic absorption in a definite spectral region (the light absorption change in the course of the reaction is recorded by a spectrophotometer), or fluorescence (the fluorescence change is continually recorded within a certain time interval using a spectrofluorimeter), etc. In other words, the choice of a method for estimating the enzymic activity is primarily determined by the possibility of reliable assessment of the substrate and reaction product concentrations.

Units of enzyme activity

- According to **the Nomenclature Committee of the International Union of Biochemistry,** to specify the activity of an enzyme, the use of the *Unit (**U**)* is recommended which is the amount of enzyme required to turn over one micromole of substrate per minute under standard conditions.
- *Specific activity* of enzyme is expressed as the amount of enzyme (in milligrams) needed to consume one micromole substrate per minute under standard conditions; its dimension is *Mmol/min·mg* protein.
- A new unit of catalytic activity, the *katal (kat*), has also been recommended, which expresses the amount of enzyme required to turn over one mole substrate per second *(mol/sec)* under standard conditions.

Regulation of enzyme activity

- The enzymes are catalysts with controlled activity. Therefore, the rates of chemical reactions in the organism can be monitored through the intermediacy of enzymes. The regulation of enzyme activity can be effected through the interaction of enzymes with various biological components or foreign compounds (for example, pharmaceuticals or toxins) which are commonly called ***enzyme modifiers, or enzyme regulators.*** The modifiers that are capable, by their action on enzymes, of accelerating reactions are called ***activators***; if the modifiers retard the reactions, they are referred to as ***inhibitors.***

Activation of enzymes.

The activation of an enzyme is defined by the acceleration of enzyme-catalyzed reactions observable after the onset of modifier action. One group of activators is made up of compounds affecting the *active center region* of an enzyme. This group includes substrates and enzyme cofactors. The cofactors (metal ions and coenzymes), while being essential structural elements of conjugated enzymes act actually as cofactors for the latter. Metal ions, coenzymes, and substrates (as precursors and active analogues of enzymes) can be used in practice as agents for activating the enzymes. The activation of certain enzymes can be finished via structural modifications thereof, leaving the active center of enzyme unaffected.

A number of approaches to such a modification may be imagined:

1. The activation of an inactive precursor referred to as proenzyme, or zymogen.
2. The activation via addition of a specific modifying group to the enzyme molecule.
3. The activation via dissociation of an inactive complex "*protein-active enzyme*".

Inhibition of enzymes.

The investigation of enzymic inhibitory reactions is important from the standpoint of practical applications for the synthesis of medicinal drugs, pesticides, etc., and in the elucidation of the mechanisms of their action. Still, a certain degree of caution should be exercised in employing the term *inhibitor*. In principle, the inhibitor is understood as an agent capable of exerting a specific deterrent action on the activity of an enzyme. The inhibitors are divided into two groups: *reversible* and *irreversible inhibitors*.

According to the mechanism of their action, the enzyme inhibitors are subdivided into the following major types:

1. competitive
2. noncompetitive
3. uncompetitive
4. allosteric
5. substrate-linked.

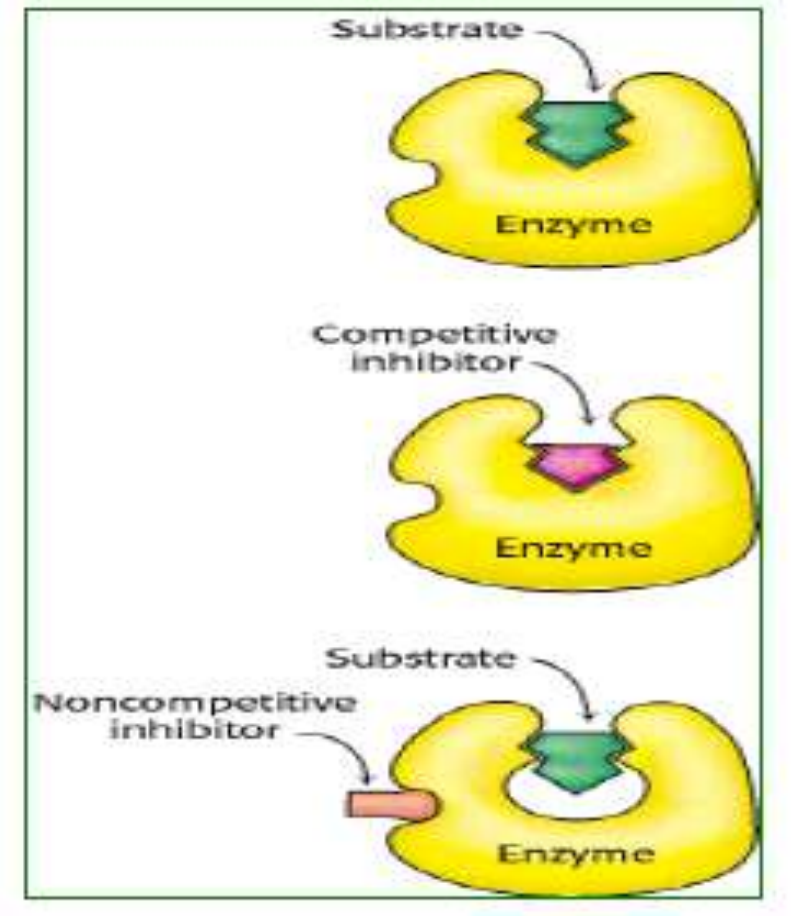
Competitive inhibition

Competitive inhibition is the enzymic reaction retardation produced by binding the enzyme active center with an inhibitor structurally related to the substrate and capable of preventing the formation of an enzyme-substrate complex. Under competitive inhibition conditions, the inhibitor and the substrate, being structurally related species, complete for the active center of enzyme. The following scheme holds true for this type of inhibition:

E + I → EI, where, **I** is the inhibitor, and **EI**, the enzyme-inhibitor complex.

The removal of inhibitory blocking can be accomplished by an excess of the substrate whose molecules eliminate the inhibitor from the active center of the enzyme molecules and reactivate the latter to catalytic activity. For example, malonic acid is a competitive inhibitor for succinate dehydrogenase.

Scheme of competitive and noncompetitive inhibition



- *Antimetabolites* are promising for use as specific pharmaceuticals. Competitive relations are possible not only between the substrate and the inhibitor, but also between the inhibitor and the coenzyme.
- *Anticoenzymes* (analogues of coenzymes, incapable of enzymic activity) likewise act as competitive inhibitors by "putting out of action" the enzyme molecules to which they are bound. Anticoenzymes (or their precursors, antivitamins) are widely used in biochemical studies and medicinal practice as effective drugs.

Noncompetitive inhibition

Noncompetitive inhibition of enzymes is the retardation associated with the effect of an inhibitor on the catalytic conversion rather than on the substrate-enzyme binding. A noncompetitive inhibitor either directly binds the catalytic groups of the enzyme active center or, on binding with the enzyme, leaves the active center free and induces conformational changes in it. The conformational changes affect the structure of the catalytic site and hinder its interaction with the substrate. Since the non-competitive inhibitor exhibits no effect on the substrate binding, in this case, as distinct from competitive inhibition, formation of a ternary complex **E-S-I** according to the scheme below is observed:

E + S + I = E-S-I

However, no conversion of this complex to any reaction products occurs. Noncompetitive inhibitors are exemplified by cyanides which are capable of strongly binding with the trivalent iron forming part of the catalytic moiety of hemin enzyme, cytochrome oxidase.

In intoxication, the toxin can be bound or eliminated from the enzyme-inhibitor complex using reactivators, or antidots. These include all SH-containing complexones (cysteine and dimercaptopropanol), citric acid, ethylenediaminetetra-acetic acid (EDTA), etc.

Allosteric regulation of enzymic activity.

- The allosteric regulation is characteristic only of a special group of enzymes with quaternary structure possessing regulatory centers for binding allosteric effectors. The negative effectors, which retard the conversion of a substrate in the enzyme active center, function as *allosteric inhibitors.* The positive effectors, on the contrary, accelerate enzymic reactions and are therefore, assigned to *allosteric activators.* Most commonly, various metabolites, as well as hormones, metal ions, and coenzymes act as allosteric effectors for enzymes. The greater the number of allosteric centers and effectors in an enzyme, the more is the enzyme responsive to metabolic alterations.
- The mechanism of action of an *allosteric inhibitor* on enzyme is effected via a change of the enzyme's active center conformation. Allosteric enzymes play an important role in the cell metabolism. They take a "key position" in metabolism, since, being extremely responsive to metabolic change they control the rate at which the materials are supplied through the whole enzymic system.

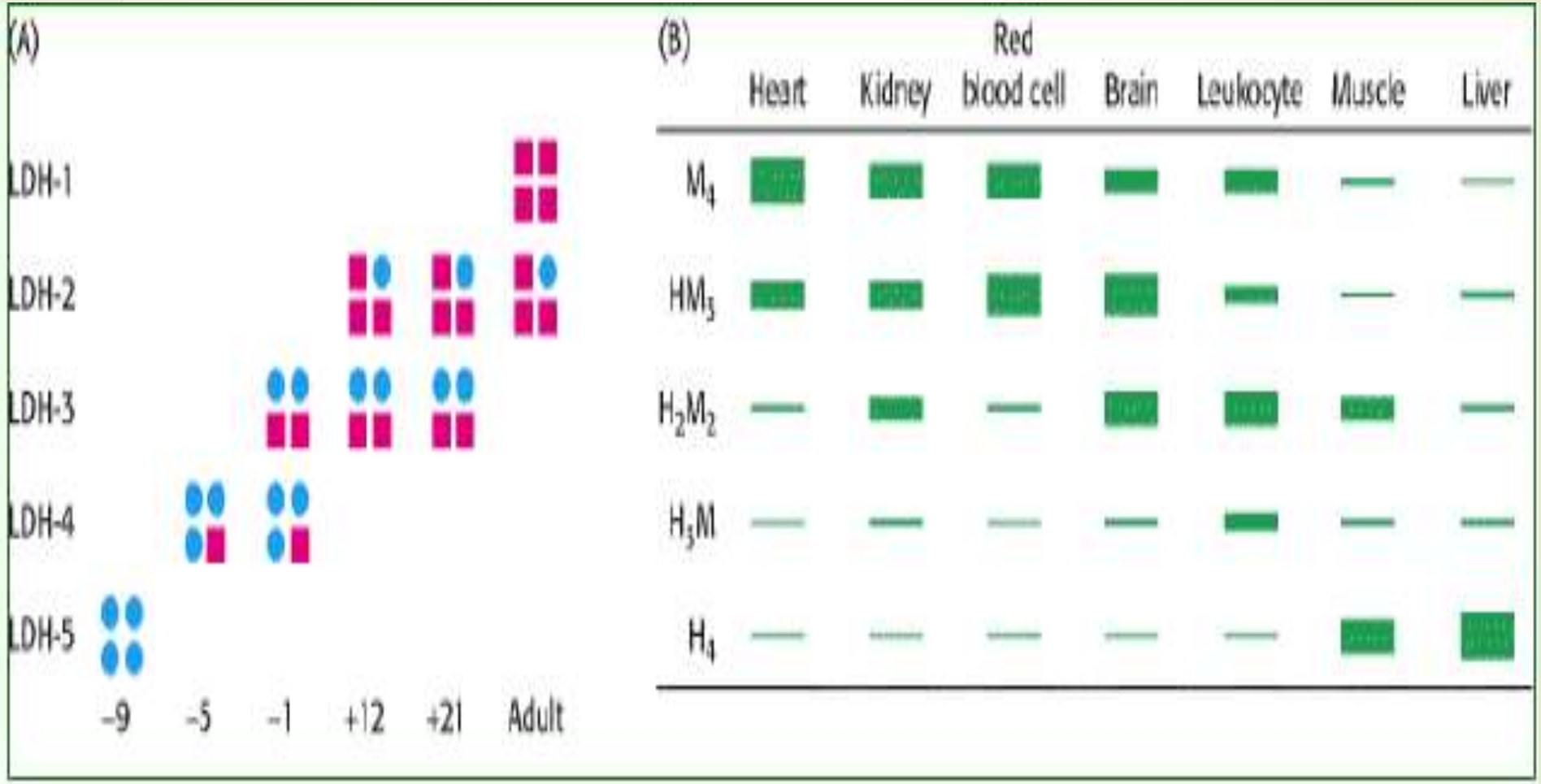
Multiple molecular forms of enzymes

A family of molecules might be predicted for the same enzyme, and the term "multiple enzyme forms" was coined to this effect. Commonly, a reference to multiple forms of enzyme implies the occurrence of enzyme proteins that differ among themselves in physico-chemical properties but can catalyze the same reaction in the organism of a definite species. Depending on their origin, multiple enzyme forms are divided into two groups:

- isoenzymes (synonym – isozymes),
- simply multiple forms of enzymes.

- Isoenzymes are molecular forms of an enzyme that arise due to *genetic* differences in the primary structure of enzyme protein, i.e. distinctions in physico-chemical properties of isoenzymes are of genetic origin. All *non genetically originated* forms of the same enzyme are referred to as *multiple enzyme forms.*
- Isoenzymes of lactate dehydrogenase are hybrids of two subunits of independent *genetic origin* (LDH1 (4H), LDH2 (3HM), LDH3 (2H2M), LDH4 (H3M) and LDH5 (4M)).
- Isoenzymes of glutamate dehydrogenase are polymers produced from subunits of the same type (different homo-polymers) – *non genetic origination*.

LDH-isoenzymes



Multi enzyme systems

- The functioning of a multienzyme system is defined by the specificity of its cellular organization. Types of multienzyme system organization may be envisaged: *functional, structure-functional*, and *combined* types.
- The *functional* organization is remarkable in that the individual enzymes are united in a function-oriented multienzyme system through the agency of metabolites that are capable of diffusing from one enzyme to another. In a functionally organized multienzyme system, the reaction product of the first enzyme in the conversion chain serves as a substrate for the second enzyme, etc.
- The *structure-functional* organization consists in that the enzymes form structural function-oriented system via enzyme-enzyme (protein-protein) interactions. Such multienzyme complexes are tightly bound and resist decomposition into constituent enzymes. This is their major distinction from functionally organized multienzyme systems. For example, the enzymes involved may become fixed on the biomembrane to form a chain. This is a pattern for the mitochondrial respiratory chain involved in energy generation and transport of electrons and protons.

Poly enzymic complex – Glycolytic enzymes

- The *combined* type of multienzyme system organization is a combination of the two above types, i.e. one part of the multienzyme system has a structural, and the other one, a functional organization. This type of organization may be exemplified by the multienzyme system of the Krebs cycle in which some of the enzymes are united into a structural complex (2-oxoglutarate dehydrogenase complex), while other enzymes are functionally interrelated through metabolite mediators.

Immobilized enzymes

Immobilized, or *insoluble*, enzymes are an artificially derived complex of soluble enzymes bound to a water-insoluble organic or inorganic carrier. The immobilization is effected by:

- physical adsorption of an enzyme onto an insoluble material,
- set-up of an enzyme in gel cells,
- covalent binding of an enzyme to an insoluble carrier,
- cross-linkage of enzyme molecules to form insoluble multienzyme complexes.

Glass, silica gel, hydroxylapatite, cellulose and its derivatives are commonly used as adsorbents.

However, immobilized enzymes are, as a rule, less active as compared with the free ones, since binding with the carrier weakens the enzyme-substrate contact.

Insoluble enzymes can readily be removed from a reaction medium; they can be washed off the reaction products and repeatedly used in further experiments.

Practical utility of enzymes

- Enzymes are widely used in practical activities of man. They are employed in various branches of agriculture and technology, let alone their exceptional importance in medical practice.
- In medicine the diagnostic importance of enzymes should be emphasized: the detection of individual enzymes in clinical analyses is an aid in the **identification of the nature of a disease**. The enzymes are used as substrates for a deficient enzyme in the organism, or as agents for the decomposition of a substrate whose excess may be thought to be the cause of a presumed disease.
- **Digestive enzymes (pepsin, trypsin, etc.)** are most commonly used in the clinic.
- **Immobilized enzymes** are used in the technological syntheses of a number of hormonal preparations, in high-sensitive analyses of drugs, in proximate analysis of biological components, and in many other applications.
- **Proteolytic enzymes** (trypsin, chymotrypsin), **immobilized** on gauze bandages or tampons, are used in surgery for cleansing purulent wounds and necrotic tissues; their action consists in enzymic degradation of dead cell proteins discharged in purulent wounds. Currently, immobilized and soluble enzymes are most commonly employed drugs of biological origin.

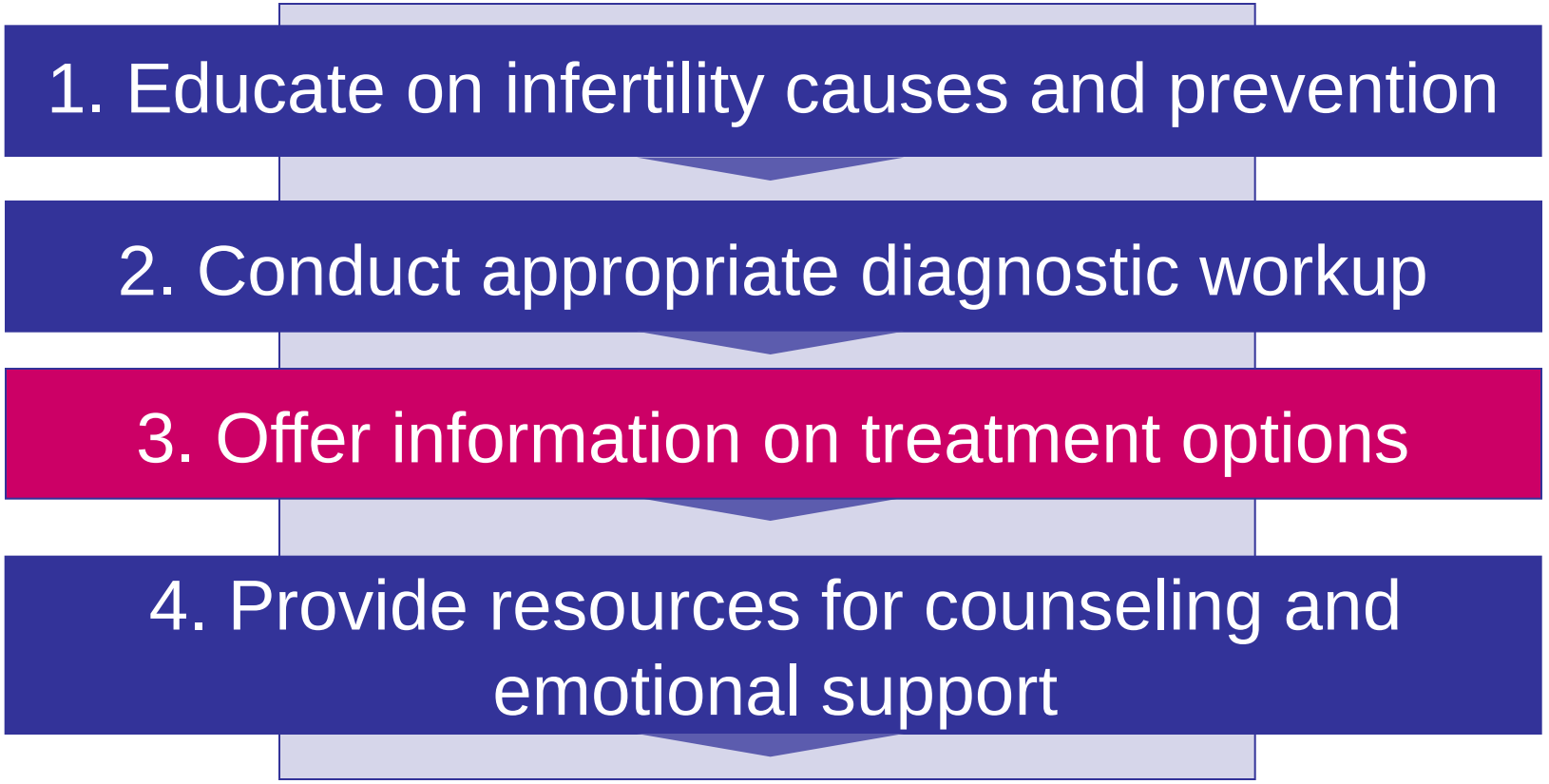
Infertility

2019

Learning Objectives

- State at least three infertility treatment options available for females.
- State at least three infertility treatment options available for males.
- List two available ovulation-inducing drugs.

Basic Services for Infertile Couples

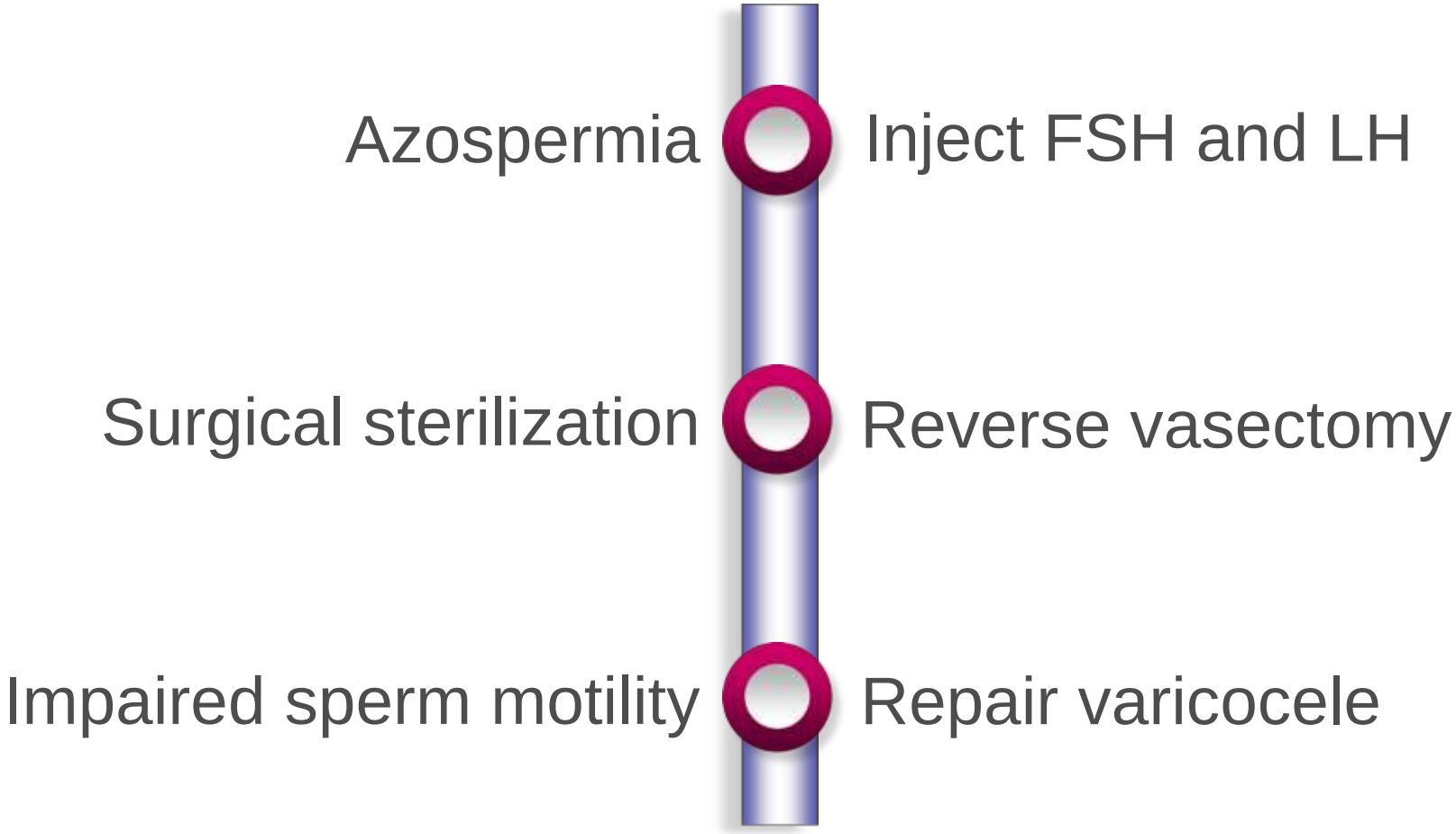


Stewart GK. 1998

Fertility Treatments Can Work

"…With thorough evaluation and application of current treatments short of IVF, embryo transfer, or GIFT, 50–60% of infertility couples will conceive."

Causes & Treatments for Male Infertility



Nelson AL, Marshall JR. 2004.

Main Causes of Female Infertility

Nelson AL, Marshall JR. 2004.

Treatments for Female Infertility

Ovulation disorders

- Maintain normal body weight
- Clomiphene
- Metformin
- Gonadotropin therapies
- Laparoscopic ovarian drilling

more…

Nelson AL, Marshall JR. 2004.

Treatments for Female Infertility
(continued)

Tubal abnormalities

- In vitro fertilization
- With or without salpingectomy
- Tuboplasty (tubal ligation reversal)

more…

Nelson AL, Marshall JR. 2004.

Treatments for Female Infertility
(continued)

Endometriosis

- Laparoscopic ablation
- Intrauterine insemination with controlled hyperstimulation
- In vitro fertilization

Nelson AL, Marshall JR. 2004.

Ovulation-Inducing Drugs: Clomiphene

- Chemically stimulates pituitary gland to produce hormones that trigger ovulation process
- Usual dosage: 50 mg/day for 5 days
- Numerous side effects
- May not be appropriate for patients with:
  - Large fibroid tumors
  - Ovarian cysts
  - Liver problems

more…

Harkness C. The Infertility Book. 1992.

Ovulation-Inducing Drugs: Bromocriptine

- Reduces production of prolactin hormone
- Dosage: 2.5 mg 1–3 times/day
- Some side effects
- May not be appropriate for patients with pituitary tumors >1 cm

Harkness C. The Infertility Book. 1992.

Ovulation-Inducing Drugs

- Human Menopausal Gonadotropins (hMG)
- Follicle-Stimulating Hormone (FSH)
  - Stimulate ovary to develop follicles
  - 75–150 IU/day (with hCG)
  - 20–40% possibility of multiple births
  - May not be appropriate in cases of pituitary tumor, ovarian cysts

more…

Harkness C. The Infertility Book. 1992.

Ovulation-Inducing Drugs (continued)

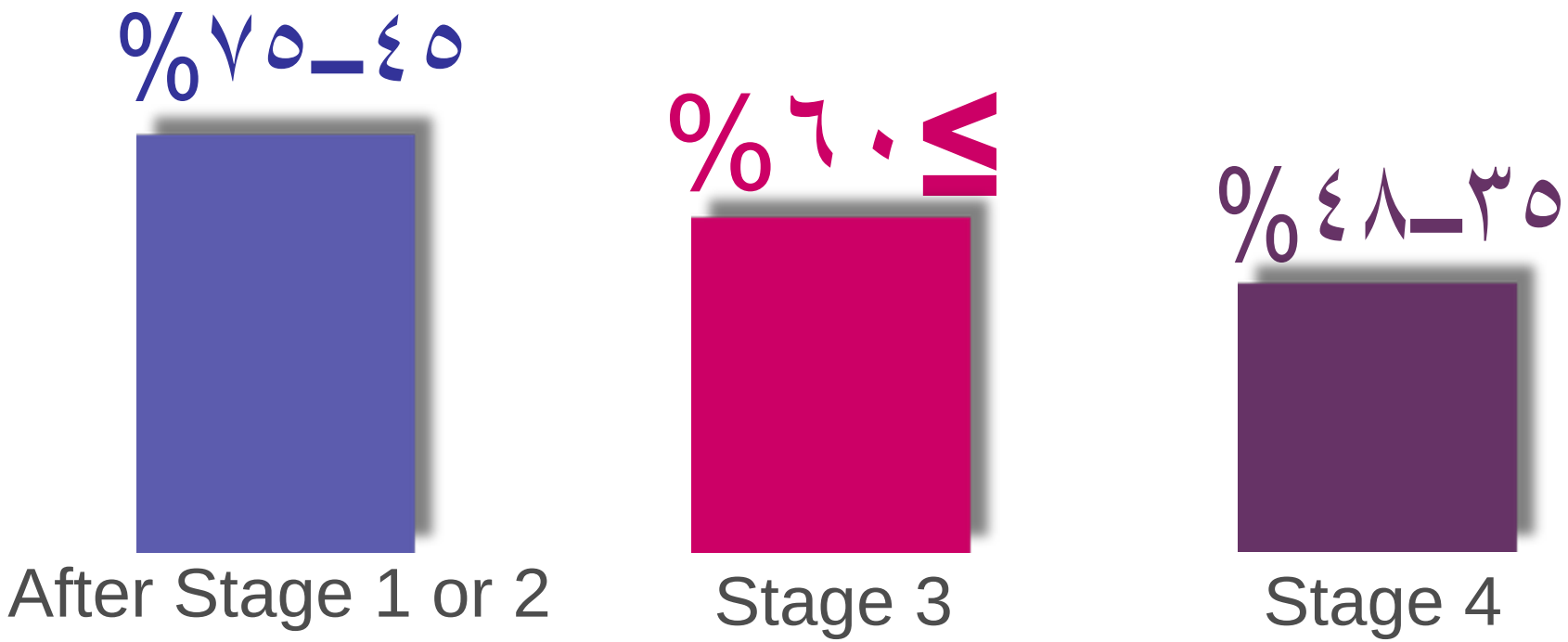
Gonadotropin-Releasing Hormone (GnRH)

- Triggers normal pituitary hormonal activity so ovulation can occur
- Effective in women with hypothalamic amenorrhea
- No known physical side effects
- Ovulation pump administers injections every 90 minutes
- User must carry pump with attached IV tubing for 1–2 weeks or until ovulation occurs

Harkness C. The Infertility Book. 1992.

Endometriosis: Treatment with Surgery

Pregnancy rates after surgery



Jennings VH, et al. 1998.

Endometriosis: Drug Therapies

- GnRH agonists
- Birth control pills

Jennings VH, et al. 1998.

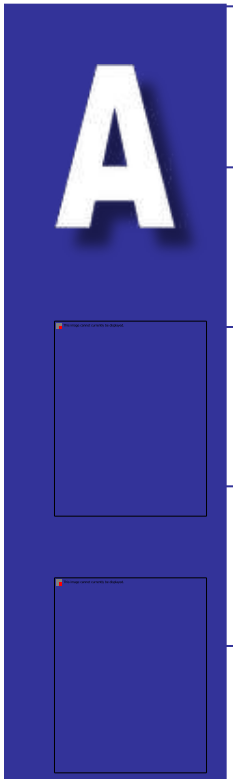
Polycystic Ovarian Disease: Treatment

Ovulation induction

- Clomiphene
- HMG, FSH, GnRH
- Insulin sensitizers (e.g. metformin, rosiglitazone)

Jennings VH, et al. 1998.

ART Treatments for Infertility



- IVF with embryo transfer
- Gamete intrafallopian transfer (GIFT)
- Zygote intrafallopian transfer (ZIFT)
- Cryopreservation
- Intracytoplasmic sperm injection (ICSI)

American Society for Reproductive Medicine. 2003.
American Society for Reproductive Medicine. 2001.

IVF with Embryo Transfer

Egg and sperm are retrieved from couple, donor(s), or both

Combined in a petri dish, incubated for 2–5 days

If fertilization and cleavage occurs, embryo is transferred through a catheter to uterus

Clapp DN. 2002.

Gamete Intrafallopian Transfer (GIFT)

Oocytes retrieved via laparoscopy

Oocytes and sperm placed in same catheter

Injected directly into the fallopian tube via laparoscopy

Embryo travels through the fallopian tube to the uterus for implantation

Clapp DN. 2002.

Zygote Intrafallopian Transfer (ZIFT)

Combines techniques used in IVF and GIFT

Ova are placed in a petri dish with sperm

If fertilization occurs, the zygote:

- Is injected into fallopian tube
- Travels through tube to uterus
- Implants in uterus

Clapp DN. 2002.

Cryopreservation

Sperm or embryos are preserved by freezing for replacement in subsequent cycles

Clapp DN. 2002.
Photo source: http://www.dcmsonline.org

Intracytoplasmic Sperm Injection (ICSI)

- A single sperm is injected directly into the cytoplasm of the oocyte
- Increases probability of fertilization

American Society for Reproductive Medicine. 2004.
Photo source: www.fertilitycentre.ca

ART Options for Same-Sex Couples

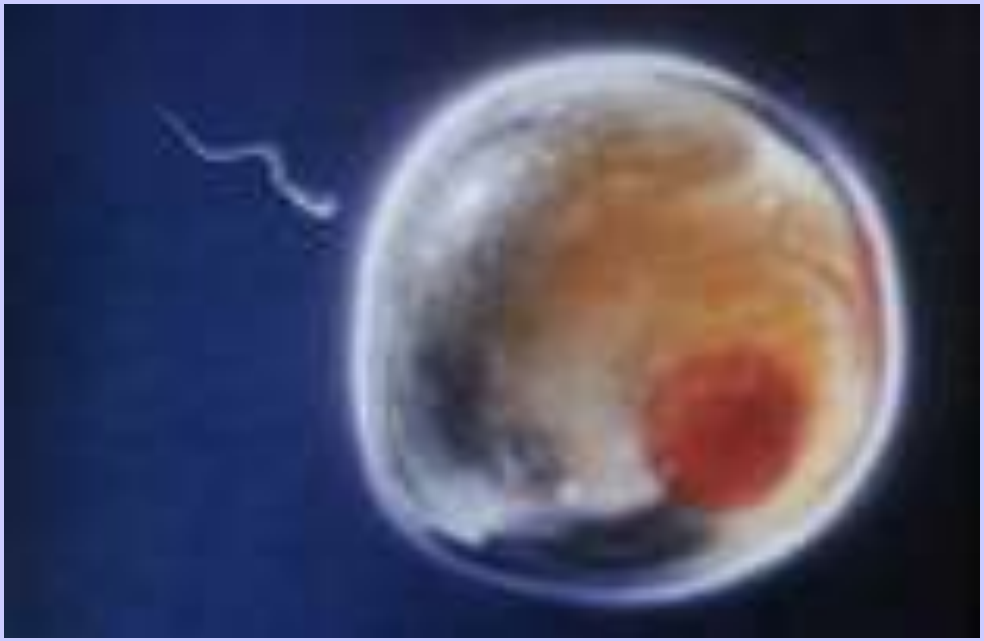
- Combination of their own and donor sperm and eggs through IVF
- Surrogacy
- Can parent biological children

Bateman S. 2004.

Pregnancy

2019

How babies come to be.



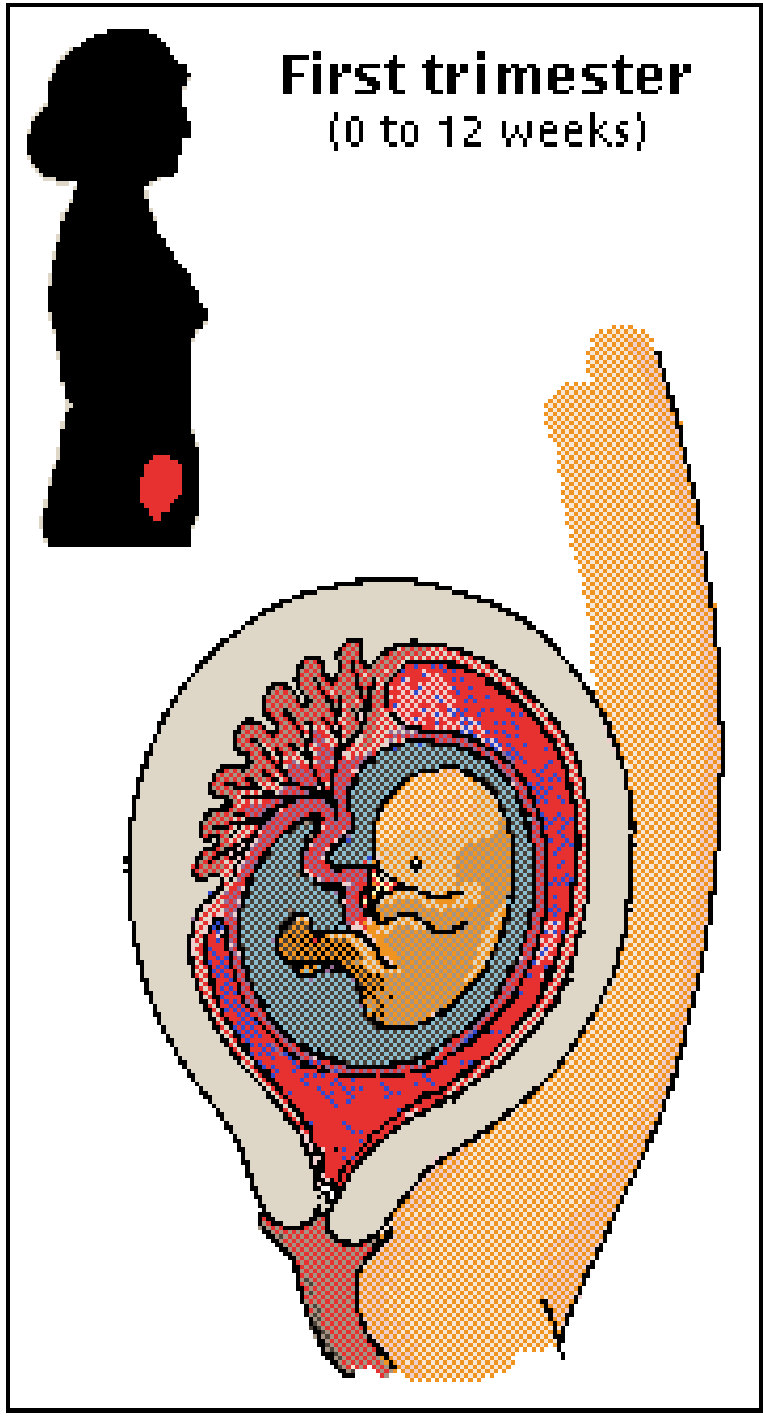
An egg goes down the fallopian tube after ovulation; if a sperm makes its way from the vagina through the uterus to the egg within 24 hours, conception is likely to occur.

Egg surrounded by sperm. A sperm penetrates the egg and conception occurs. It is called a zygote until it reaches the uterus in 3-4 days.

Signs and Symptoms Pregnancy – 40 weeks

- A missing menstrual period
- Morning sickness and nausea
- Frequent urination
- Cravings
- Breasts enlarge
- Fatigue and dizziness

A simple urine test from the doctor will show whether or not a woman is pregnant.



The embryo may float freely in the uterus for about 48 hours before implanting.

Upon implantation, complex connections between the mother and embryo develop to form the placenta.

Call the doctor if any of the following occur:

- Vaginal bleeding
- Sharp abdominal pain or cramping
- Loss of fluid from the vagina
- Severe or prolonged nausea or vomiting
- Frequent dizzy spells
- Painful urination
- High fever over 100 degrees F.
- Vaginal discharge that is irritating

Call the doctor if any of the following occur:

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- Frequent dizzy spells
- Painful urination
- High fever over 100 degrees F.
- Vaginal discharge that is irritating

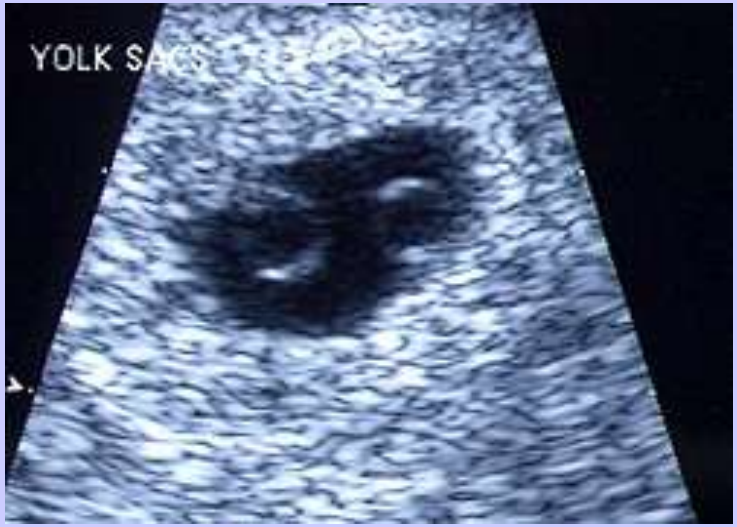
Other considerations:

- No medications, including over-the counter drugs, unless approved by your doctor.
- No drugs or alcohol.
- No x-rays.
- No saunas and hot tubs.
- No vaccinations during pregnancy.
- Avoid cats, cattle, sheep and pigs. They have a parasite that causes toxoplasmosis.

X-Ray

1 Month

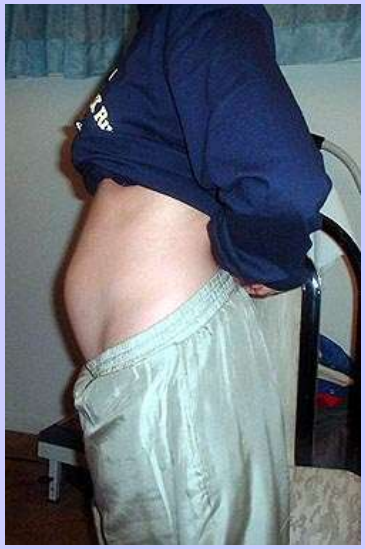
By the end of the first month, the embryo is about 1/4 of an inch long. The heart, no larger than a poppy seed, has begun beating. Head, mouth, liver, and intestines begin to take shape.

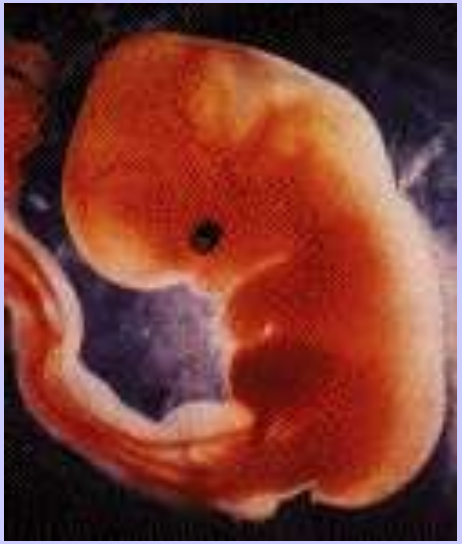


twins

2 Months

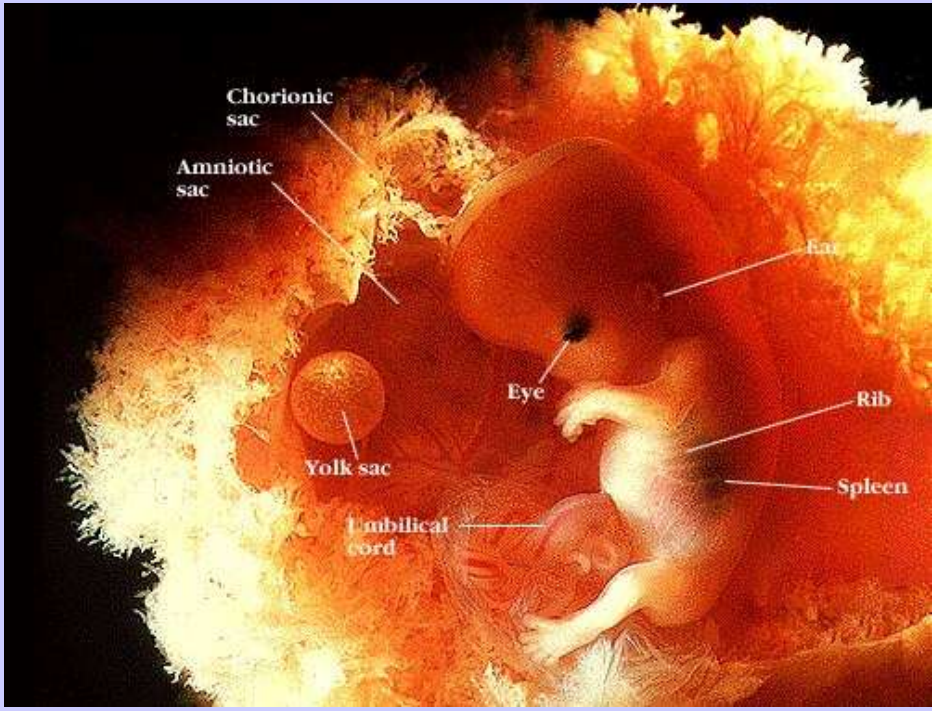
The embryo grows to about an inch long and has distinct, slightly webbed fingers. Veins are clearly visible. The placenta is already nourishing the baby through the umbilical cord. The heart has divided into right and left chambers. Veins are clearly visible. Most vital organs are developed.



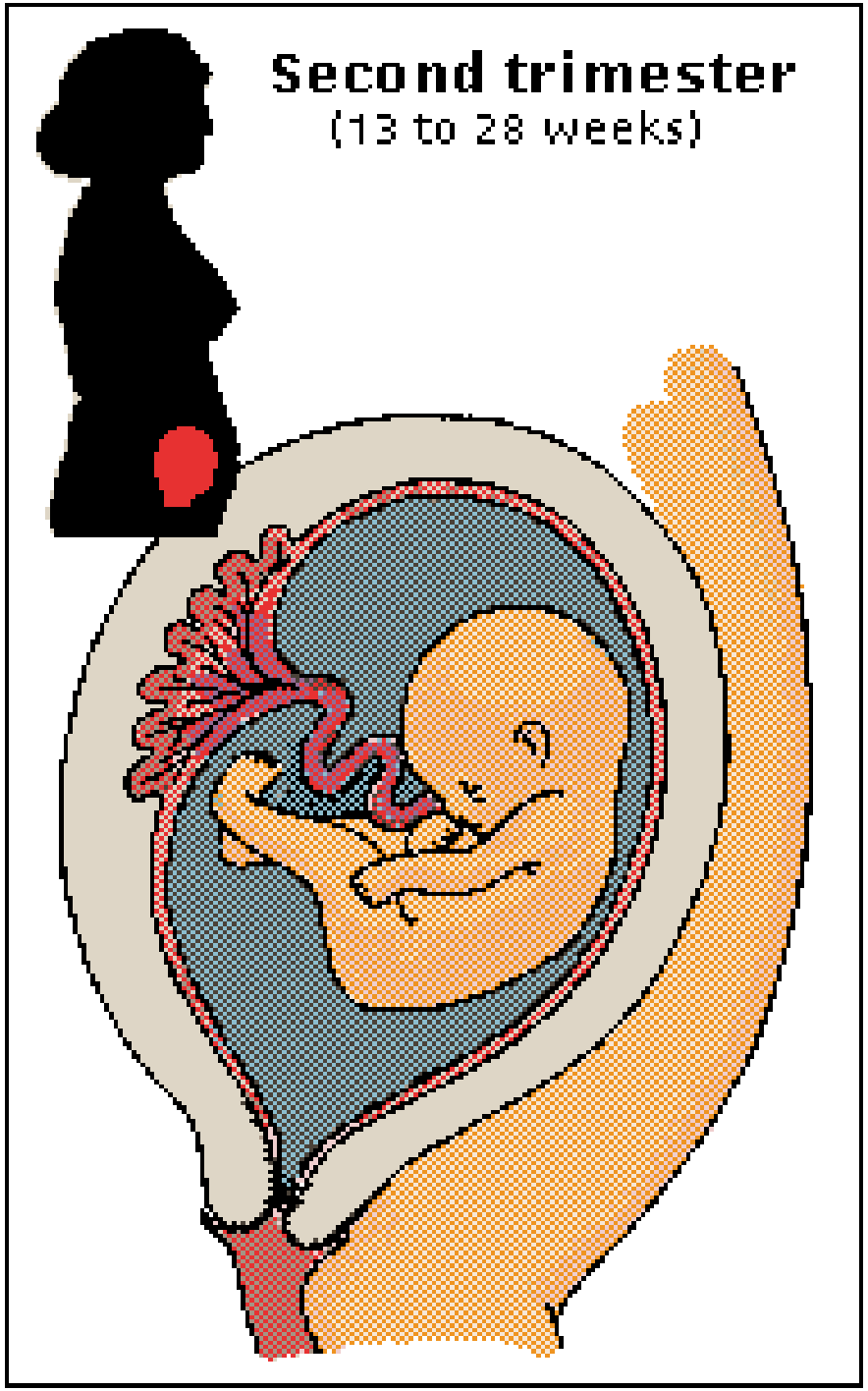


3 Months

Starting at eight weeks, your baby is called a fetus. By the end of the first trimester, the fetus is 2 1/2 to 3 inches long and is fully formed. He has begun swallowing and kicking. All organs and muscles have formed and are beginning to function. The arms, legs, hand, and fingers are fully developed. The nails on his fingers and toes are starting to develop.

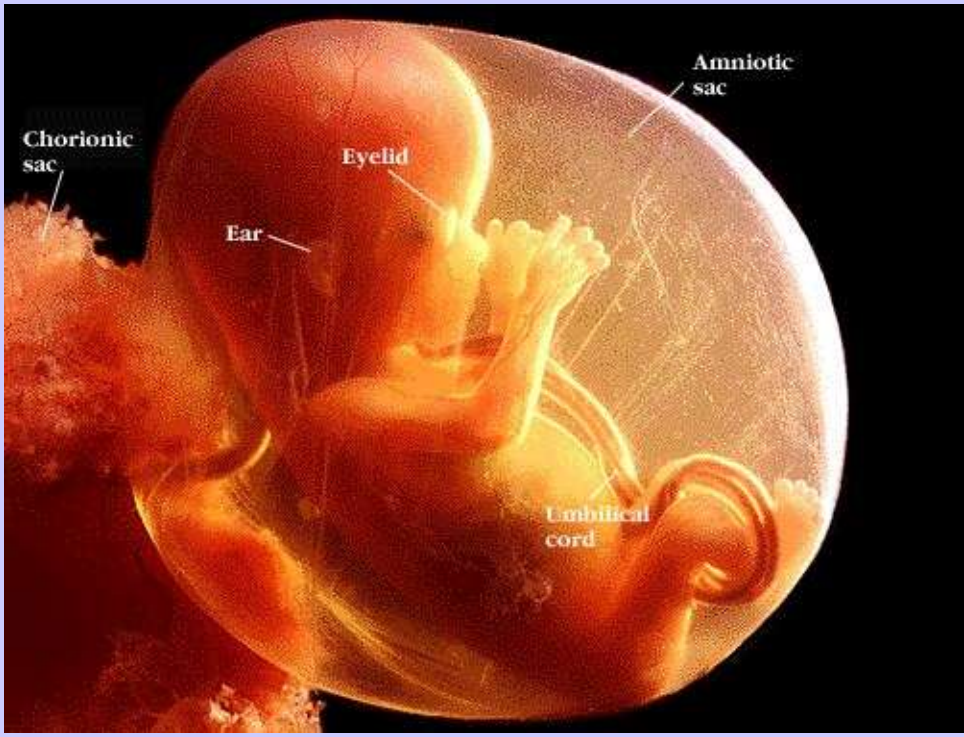


- Skin – Every woman's body reacts differently to pregnancy.
  - Oily, dry or scaly
  - Stretch marks
  - Facial skin may darken
- Emotions
  - Need a few extra breaks or time to relax.
  - May experience mood swings, depression and bad dreams.
- Feel baby's movements



4 Months

Your baby is about 2 inches long and is covered with a layer of thick, downy hair called lanugo. The first outlines of the face are showing. His heartbeat can be heard clearly. This is when many mothers feel their baby's first thrilling kick.

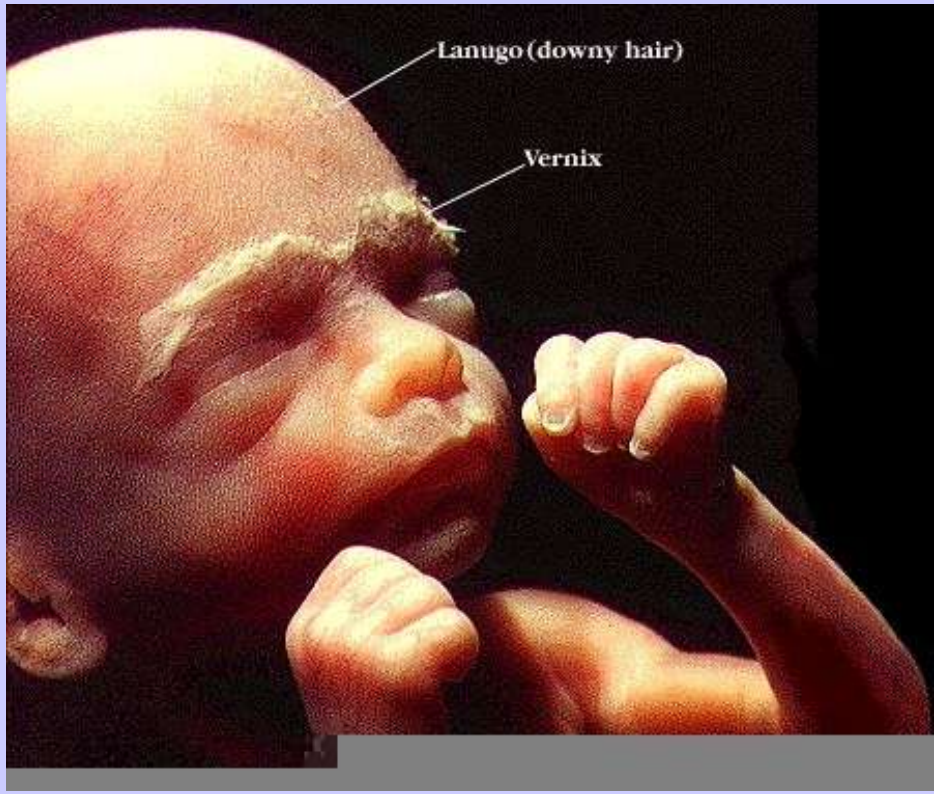


5 Months

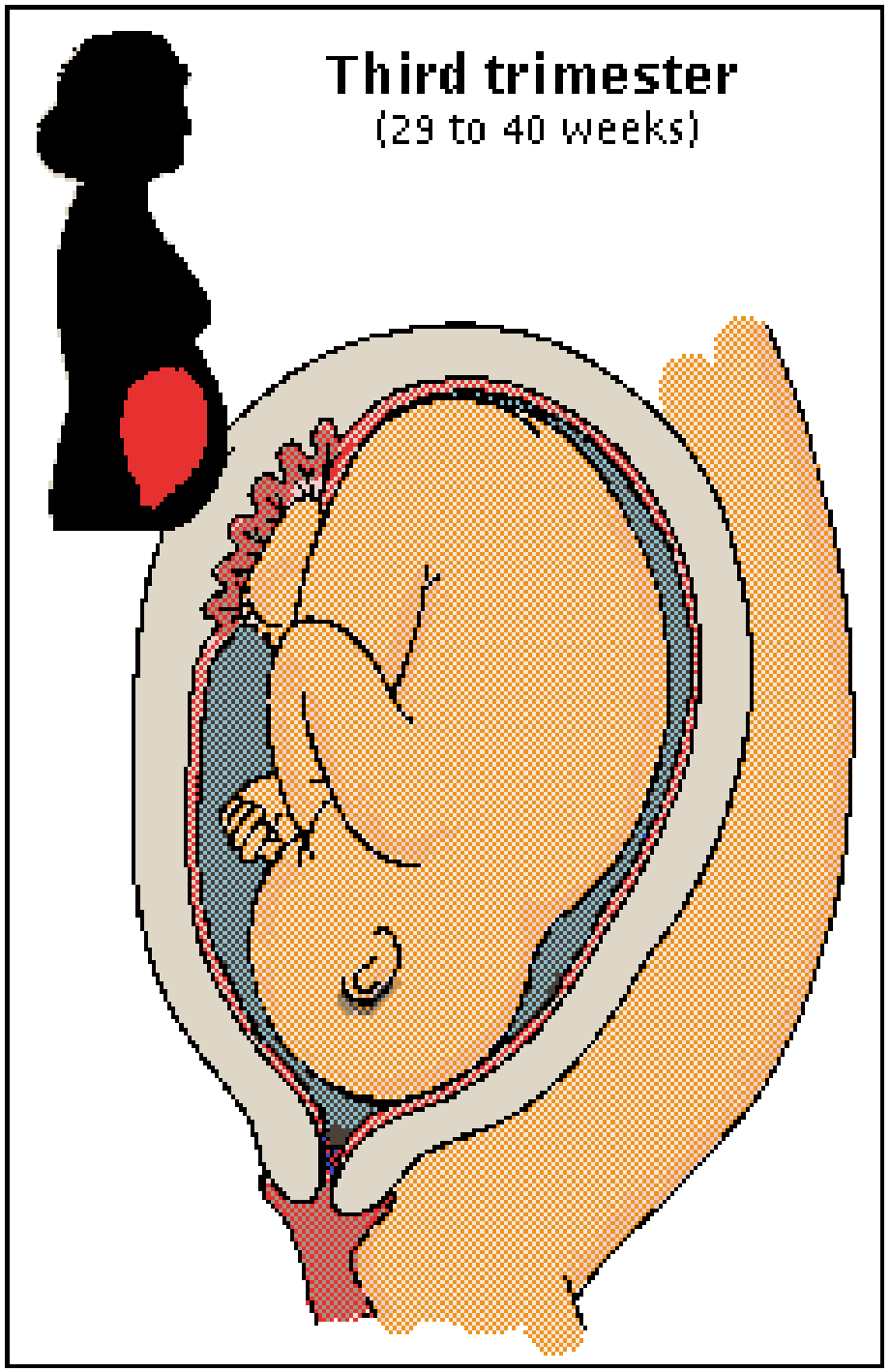
If you have an ultrasound, you might see him sucking his thumb. By the end of this month, your baby will be nearly 8 inches long and weigh almost a pound. The skull bones are the most important bones being developed at this time.

6 Months

Tiny eyebrows and eyelids are visible. There is a lot of evidence to show that the baby can hear the outside world. Your baby's lungs are filled with amniotic fluid, and he has started to practice breathing movements. If you talk or sing, he can hear you. Fingerprints are formed.



- Abdomen enlarges
- Fatigue is common
- Expectant fathers take more interest because they can feel the baby move.
- Baby moves a lot
  - A mother should feel the baby move every couple of hours. If not, she should call her doctor.

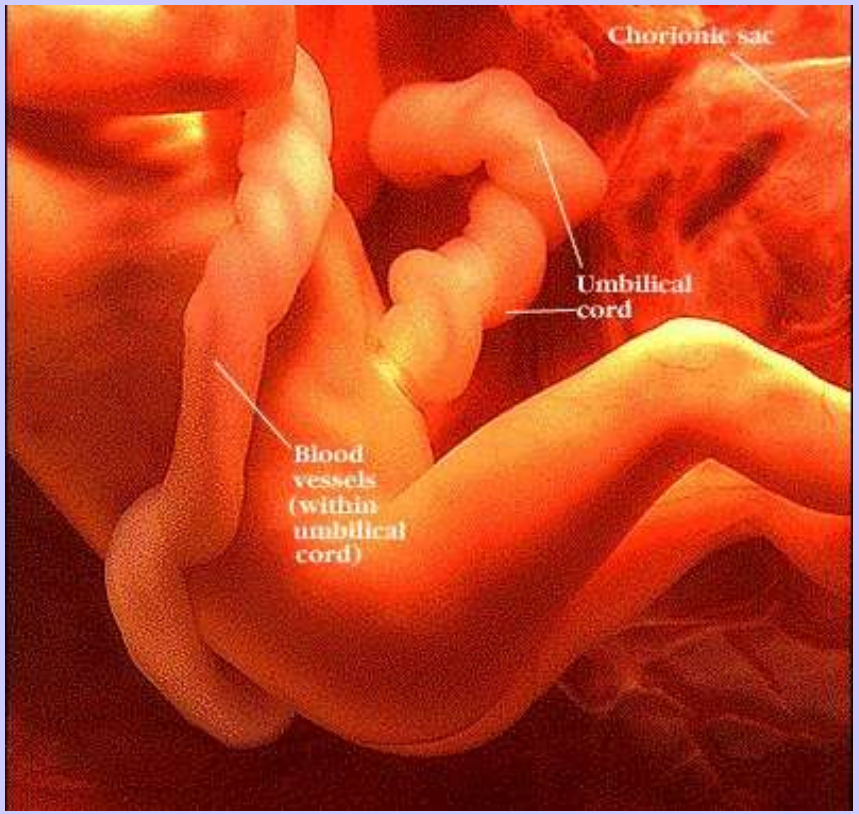


Discomforts that might be experienced

- Heartburn
- Shortness of breath
- Heart palpitations
- Leg cramps
- Round ligament pains

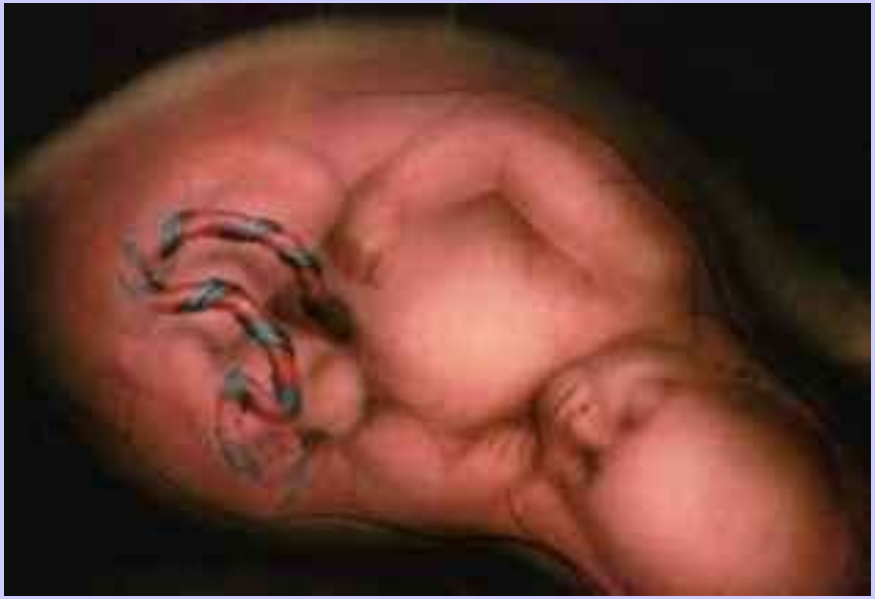
7 Months

By the end of the seventh month, your baby weighs about 3 1/2 pounds and is about 12 inches long. His body is well formed. Fingernails cover his fingertips. He may try to turn toward a source of bright light.



8 Months

Your baby is gaining about half a pound per week, and layers of fat are piling on under his skin. He has probably turned head-down in preparation for his coming birth. He weighs between 4 and 6 pounds.



Your baby is a hefty 6 to 9 pounds and measures somewhere between 19 and 22 inches. The lungs develop in preparation fro breathing and the head is now head-down. As he becomes more crowded, you may feel him move around less. The last few weeks, the baby "drops" in preparation for delivery – giving the mother a little breathing space.

9 Months

Danger Signals

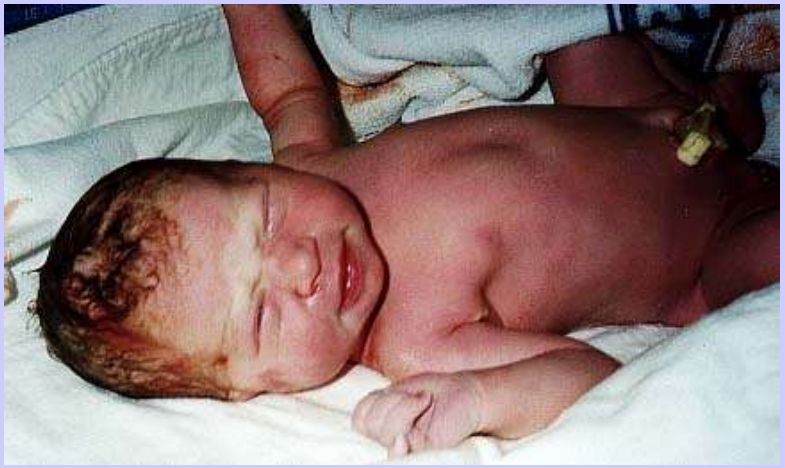
Call the doctor if any of these problems occur.

- Vaginal bleeding
- Sharp abdominal pain/cramping
- Loss of fluid
- Frequent dizzy spells
- Visual disturbances
- Nausea or vomiting
- Sudden and excessive swelling of face, hands, and feet
- Headache
- Burning, painful urination
- Fever
- Vaginal discharge

Weight gain during pregnancy
25-30 pounds

- Baby – 7 ½ pounds
- Placenta – 1 ½ pounds
- Uterus – 2 pounds
- Amniotic fluid – 1 ½ pound
- Extra blood and water – 4 ½ pounds
- Breast tissue – 3 pounds
- Maternal stores of protein – 4 pounds

Baby's Arrival



Pregnancy Issues

- Toxemia
- Ectopic Pregnancy
- Stillborn
- Spontaneous Abortion