Introduction to Clinical Biochemistry

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The clinical biochemistry laboratory

- Biochemical laboratory plays only a part in the overall assessment and management of the patient.
- Biochemical instruments are available in most hospital.
- All clinical biochemistry laboratories provide examinations for urgent tests, and can speed up the analysis of some samples more quickly than others

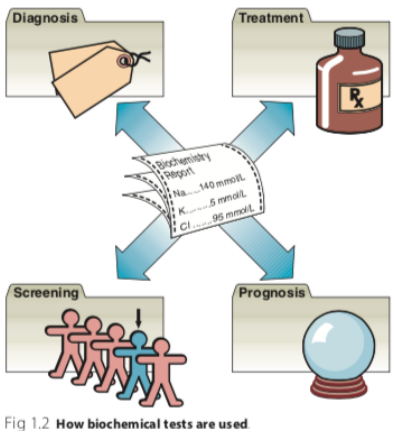

Fig 1.2 **How biochemical tests are used**

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The use of the laboratory

- Every biochemistry analysis should attempt to answer a question that the clinician has posed about the patient.
- what to do?
- Specimen collection.
- Sampling errors !
- Timing !
- Analysing the specimen
- Unnecessary testing!

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Specimen collection, blood

- There are mainly two types of specimens that are used in biochemical analysis:
- Such as blood; serum and plasma, urines.
- If blood is collected into a plain ‘’normal test tube’’ which allows to clot, then after centrifugation a *serum* specimen is obtained.
- In other cases, the blood is collected into a tube containing an anticoagulant such as heparin; (anticoagulant does not allow to clot).

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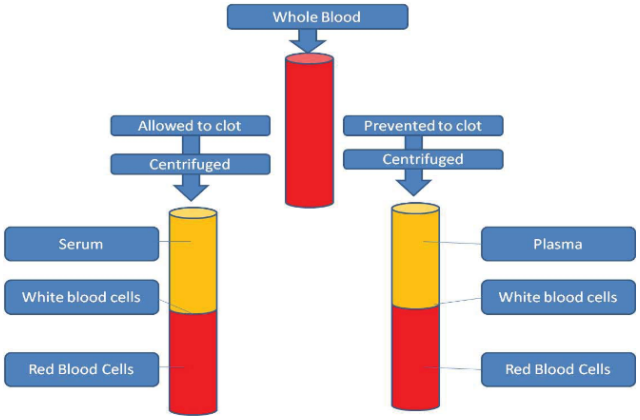
What is the difference between serum and plasma?

- When blood is centrifuged, the supernatant is called *plasma*, but contains the anticoagulant as well.
- However, there are several differences between serum and plasma.
- As follow:



The difference between plasma and serum

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What is the difference between serum and plasma? In term of composition

Characteristics	Serum	Plasma
Composition	Serum contains proteins, electrolytes, antibodies, antigens and hormones.	Plasma is considered as the medium of blood in which RBCs (Red Blood Cells), WBC (White Blood Cells) and other components of blood are suspended.
Composition (Antibodies)	Serum contain antibodies and cross react with recipient antigen.	Blood plasma contains antibodies, a type of protein that can fight a substance considered foreign to the host body.
Composition	Serum contains proteins like albumin and globulins.	Plasma contains the clotting factors and water.
Fibrinogen	Absent	Present
Composition (Water)	Serum contains 90% water.	Plasma contains 92-95% water.
Storage	Serum can be stored at 2-6 degrees centigrade for several days.	Frozen plasma can be stored for up to a year.

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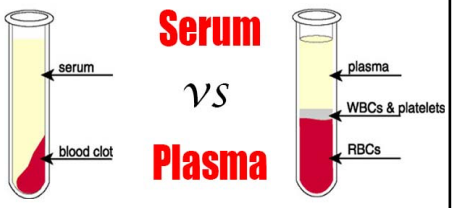
What is the difference between serum and plasma? In term of use

Characteristics	Serum	Plasma
Uses	Serum is used for various diagnostic tests used for determining the levels of hCG, cholesterol, proteins, sugar, etc., in the blood.	Plasma aids in the maintenance of blood pressure and in the regulation of body temperature



Serum = Plasma – Clotting Factors

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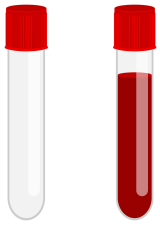
Which types of tubes that are used to collect blood samples?

- For each test, an appropriate tube should be used to collect the blood.
- If a sample is collected into the wrong container, it should never be decanted into another type of tube.
- For example, samples for glucose should be collected into a special container containing fluoride, which inhibits glycolysis; otherwise the time taken to deliver the sample to the laboratory can affect the result.



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Which types of tubes that are used to collect blood samples?

- For example, blood that has been exposed, even briefly, to EDTA (an anticoagulant used in sample containers for lipids) will have a markedly reduced calcium concentration till become zero, but potassium concentration becomes higher.
- This is because EDTA is a chelator of calcium and is present as its potassium salt.
- See next page types of tubes used for blood collection.

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Which types of tubes that are used to collect blood samples?

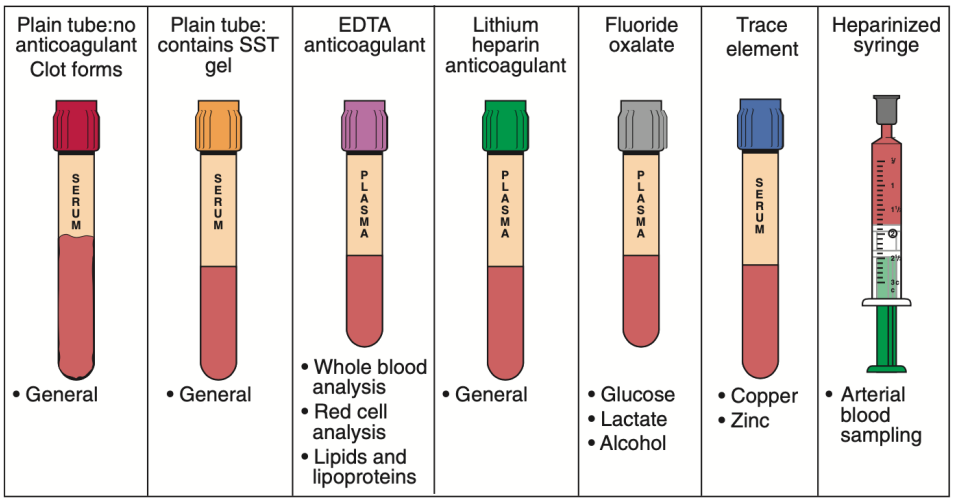

Fig 2.1 **Blood specimen tubes for specific biochemical tests.** The colour-coded tubes are the vacutainers in use in the authors' hospital and laboratory.

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Specimen collection; Urine

- Urine specimen containers may include an inhibitor of bacterial growth, or acid to stabilize certain metabolites.
- They need to be large enough to hold a full 24-hour collection.
- Random urine samples are collected into small 'universal' containers.

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Other types of biochemical specimens?

Table 1.1 **The clinical biochemistry repertoire**

Core biochemical tests

- Sodium, potassium and bicarbonate
- Urea and creatinine
- Calcium and phosphate
- Total protein and albumin
- Bilirubin and alkaline phosphatase
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST)
- Free thyroxine (FT_4) and Thyroid Stimulating Hormone (TSH)
- γ-glutamyl transpeptidase (γGT)
- Creatine kinase (CK)
- H^+, PCO_2 and PO_2 (blood gases)
- Glucose
- Amylase

Specialized tests

- Hormones
- Specific proteins
- Trace elements
- Vitamins
- Drugs
- Lipids and lipoproteins
- Intermediary metabolites
- DNA analyses

Other specimens

Table 2.1 **Specimens used for biochemical analyses**

- Venous blood, serum or plasma
- Arterial blood
- Capillary blood
- Blood spot on a filter paper (Guthrie Card)
- Urine
- Faeces
- Cerebrospinal fluid (CSF)
- Sputum and saliva
- Tissue and cells
- Aspirates, e.g.
  - pleural fluid
  - ascites
  - joint (synovial) fluid
  - intestinal (duodenal)
  - pancreatic pseudocysts
- Calculi (stones)

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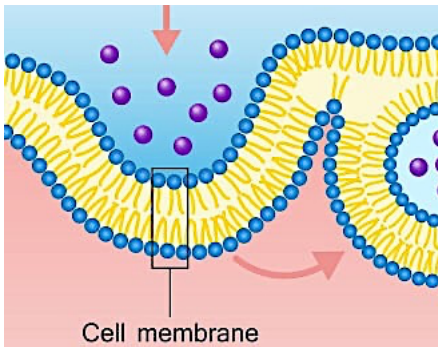
Other important factors could affect blood test?

- ***Incorrect specimen storage.***
- A blood sample stored overnight will show falsely high potassium, phosphate and red cell enzymes, such as lactate dehydrogenase, because of leakage into the extracellular fluid from the cells.
- ***Timing:*** in the sample collection and in the tests preforming.



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Be careful with dangerous specimens !

- All specimens from patients with dangerous infections should be labelled with a yellow 'dangerous specimen' sticker.
- A similar label should be attached to the request form.
- Of most concern to the laboratory staff are hepatitis B and H IV.

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Analysing the specimen

- Samples must be well-labelled.
- Never mix up samples.
- Once the results are available they are collated and a report is issued.
- Start centrifugation if required.
- Prepare the protocols ‘’procedures’’ and the kits to start tests.
- Do you need an incubators? 37˚C.

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The interpretation of results

- **How biochemical results are expressed?**
- Results are reported as concentrations, usually in terms of the number of moles in one litre (mol/L).
- Also, the can be expressed in unis of mg/dl (dl=100 ml)

Mole	Abbreviation	Definition
Millimole	mmol	$\times 10^{-3}$ of a mole
Micromole	µmol	$\times 10^{-6}$
Nanomole	nmol	$\times 10^{-9}$
Picomole	pmol	$\times 10^{-12}$
Femtomole	fmol	$\times 10^{-15}$

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Concentration in Molar units

- The concentration of any analyte in a body compartment is a ratio: the amount of the substance dissolved in a known volume. Changes in concentration can occur for two reasons:
- 1- The amount of the analyte can increase or decrease.
- 2- The volume of fluid in which the analyte is dissolved can similarly change.

Glucose (blood) *Normal range=* 4.0–5.5 mmol/L (divide by 0.05551 to convert to mg/dL)

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Reference ranges or Reference intervals

Table 5.1 **Alphabetical list of reference intervals – general**

(All reference intervals listed are for serum measurements in adults unless otherwise stated)

Alanine aminotransferase (ALT)	3–55 U/L
Albumin	35–50 g/L
Alkaline phosphatase (ALP)	30–130 U/L
Aspartate aminotransferase (AST)	12–48 U/L
Amylase	70–300 U/L
Bicarbonate	22–29 mmol/L
Bilirubin (total)	<21 µmol/L
Calcium (adjusted)	2.2–2.6 mmol/L
Chloride	95–108 mmol/L
Cholesterol (total plasma)	<5 mmol/L (divide by 0.02586 to convert to mg/dL)
C-reactive protein (CRP)	0–10 mg/L
Creatine kinase (CK)	40–320 U/L (males) 25–200 U/L (females)
Creatinine	40–130 µmol/L
γ-glutamyl transpeptidase (γGT)	<36 U/L
Glucose (blood)	4.0–5.5 mmol/L (divide by 0.05551 to convert to mg/dL)
Glycated haemoglobin (HbA_{1c})	6–7% (42–53 mmol/mol Hb) taken to indicate good diabetic control
Hydrogen ion (H^+)(arterial blood)	35–45 nmol/L
Iron	10–40 µmol/L
Transferrin percentage saturation	<50% (females) <55% (males)
Lactate	0.7–1.8 mmol/L
Lactate dehydrogenase (LDH)	230–525 U/L
Magnesium	0.7–1.0 mmol/L
Osmolality	275–295 mmol/kg (serum) 50–1400 mmol/kg (urine)
PCO_2 (arterial blood)	4.6–6.0 kPa
pH (arterial blood)	7.35–7.45
Phosphate	0.8–1.5 mmol/L
PO_2 (arterial blood)	10.5–13.5 kPa
Potassium	3.5–5.3 mmol/L
Total protein	60–80 g/L
Sodium	133–146 mmol/L
Triglyceride	<2.5 mmol/L
Urate	200–430 µmol/L (males) 140–360 µmol/L (females)
Urea	2.5–7.8 mmol/L

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Laboratory analytical performance !

- A number of terms describe biochemical results.
- These include:

1. Precision and accuracy.
2. Sensitivity and specificity
3. Quality assurance.
4. Reference intervals.

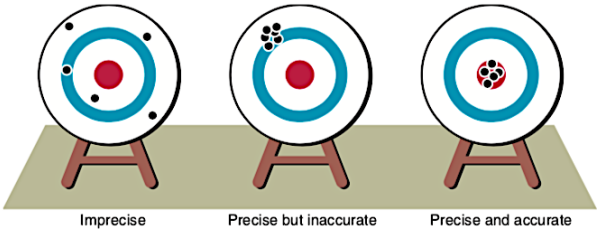

Fig 3.2 **Precision and accuracy.**

- What is it? A list of all parameters that we measure in biochemistry lab. See the list?

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Biological factors affecting the interpretation of results

- The discrimination between normal and abnormal results is affected by various physiological factors that must be considered when interpreting any given result.
- These include:

1. Sex: Reference intervals for some analytes such as serum creatinine are different for men and women.
2. Age: There may be different reference intervals for neonates, children, adults and the elderly.
3. Diet: The sample may be inappropriate if taken when the patient is fasting or after a meal.
4. Timing: There may be variations during the day and night.
5. Stress and anxiety; These may affect the analyte of interest.

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Continue ….

6. Positions of the patient.
7. Effects of exercise: Strenuous exercise can release enzymes from tissues.
8. Medical history: Infection and/or tissue injury can affect biochemical values.
9. Pregnancy; This alters some reference intervals.
10. Menstrual cycle: Hormone measurements will vary throughout the menstrual cycle.
11. Drug history; Drugs may have specific effects on the plasma concentration of some analytes.

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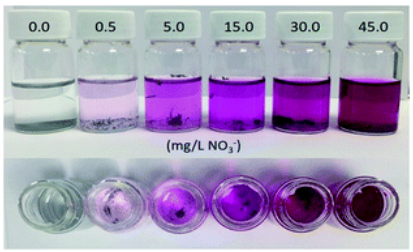
What are the common biochemical techniques used for examinations?

- Colorimetric methods.
- Kinetics methods.
- In both: we use Spectrophotometer.



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Colorimetric methods using Spectrophotometry.

- Colorimetry, **measurement of the wavelength (nm)and the intensity of electromagnetic radiation in the visible region of the spectrum**.
- It is used for determination of concentrations of substances that absorb light.
- Follows the beer-lambert law?

$$A = \varepsilon c l$$

A	Absorbance	
ε	Molar absorption coefficient	$M^{-1}cm^{-1}$
c	Molar concentration	M
l	optical path length	cm

$$T = \frac{I}{I_0} \qquad A = \log_{10}\frac{I_0}{I} \qquad A = -\log_{10} T$$

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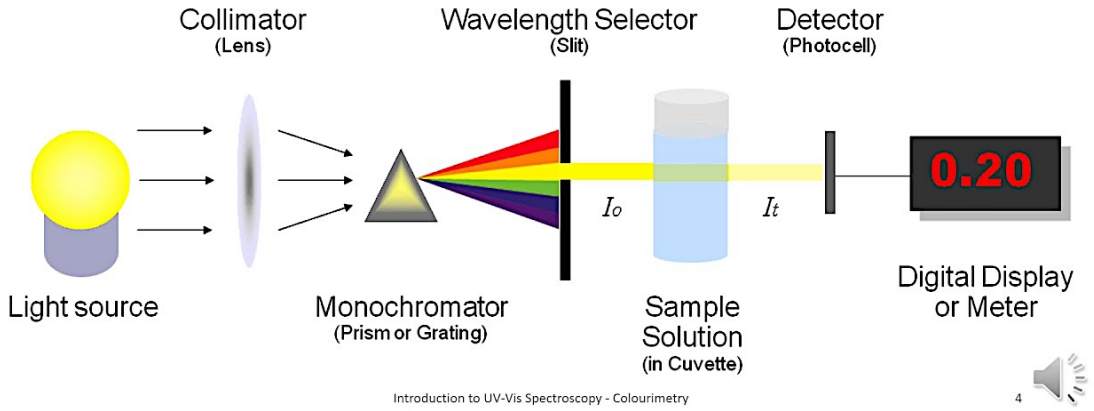
Basic of colorimeter



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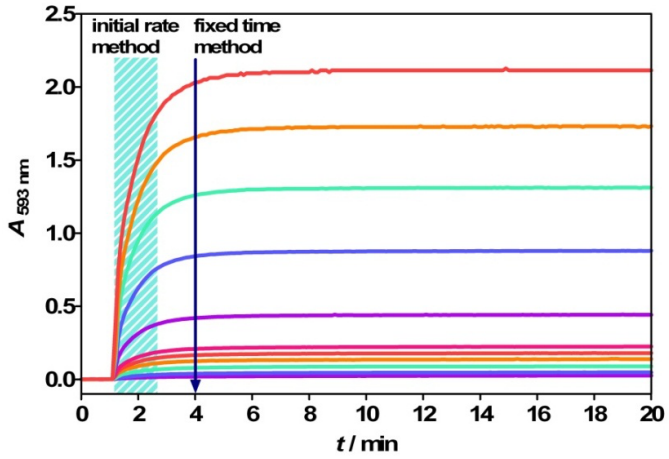
Kinetics methods.

- Follow the rate of the reaction (absrbance) in term of time!



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Glucose determination and Diabetes Mellitus

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Glucose metabolism and diabetes mellitus

- Glucose is the carbohydrate fuel of the body, all other carbohydrates being converted to glucose after digestion and absorption.
- Insulin controls blood glucose by promoting the storage of metabolic fuels.

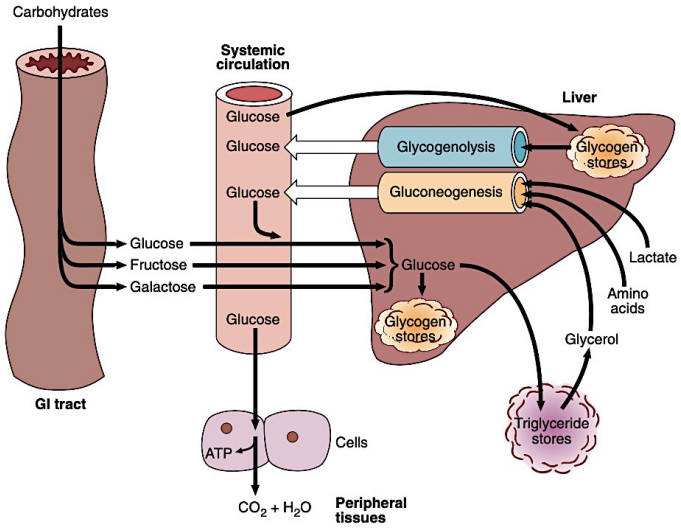

Fig 31.1 **Glucose homeostasis.**

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Diabetes mellitus

- Diabetes mellitus is characterized by hyperglycaemia, absolute or relative insulin lack and late complications.
- Type 1 diabetes mellitus is caused by a complete lack of insulin and is most common in the young.
- Type 2 diabetes mellitus accounts for 85% of all diabetics and can occur at any age.

3

More details….

- Diabetes is a disease in which blood glucose levels are above normal (abnormal).
- People with diabetes have problems on converting food to energy.
- After a meal, food is broken down into a sugar called glucose, which is carried by the blood to cells throughout the body.
- Cells use insulin, a hormone made in the pancreas, to help them convert blood glucose into energy.

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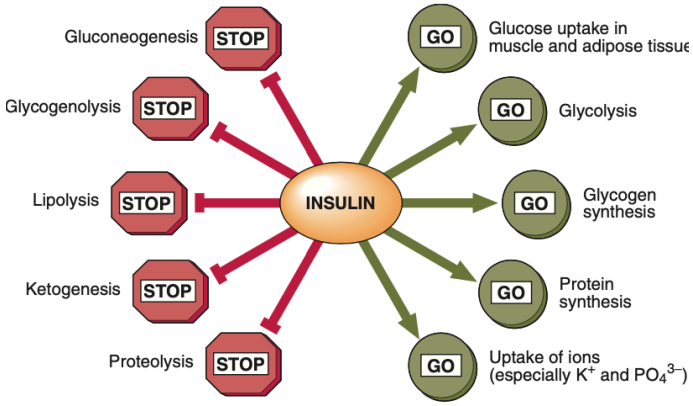
What does insulin affect by?

- The effects of insulin are opposed (work against) by other hormones, e.g. glucagon, adrenaline, glucocorticoids and growth hormone.



31.2 **The actions of insulin.**

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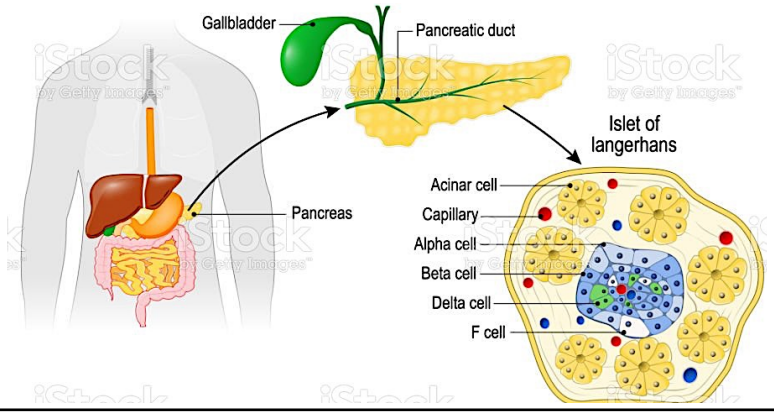
Why People develop diabetes?

- People develop diabetes because, the pancreas does not make enough insulin or because, the cells in the muscles, liver, and fat do not use insulin properly, or both.
- As a result, the amount of glucose in the blood increases while the cells are starved of energy.



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Hyperglycemia or hypoglycemia

- Over the years, high blood glucose, also called hyperglycemia, damages nerves and blood vessels, which can lead to complications such as heart disease and stroke, kidney disease, blindness, nerve problems, gum infections, and amputation.
- However, hypoglycemia a low blood glucose when there is insufficient concentration of glucose.

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Diabetes mellitus

- Diabetes mellitus is the commonest endocrine **disorder** faced in clinical practice (hospital).
- It may be defined as a **syndrome** characterized by hyperglycaemia due to an insulin resistance and a lack (decrease) of insulin.
- Primary diabetes mellitus is generally sub classified into Type 1 or Type 2.
- Secondary diabetes mellitus may result from pancreatic disease, endocrine disease such as Cushing's syndrome, drug therapy, and, rarely, insulin receptor abnormalities.

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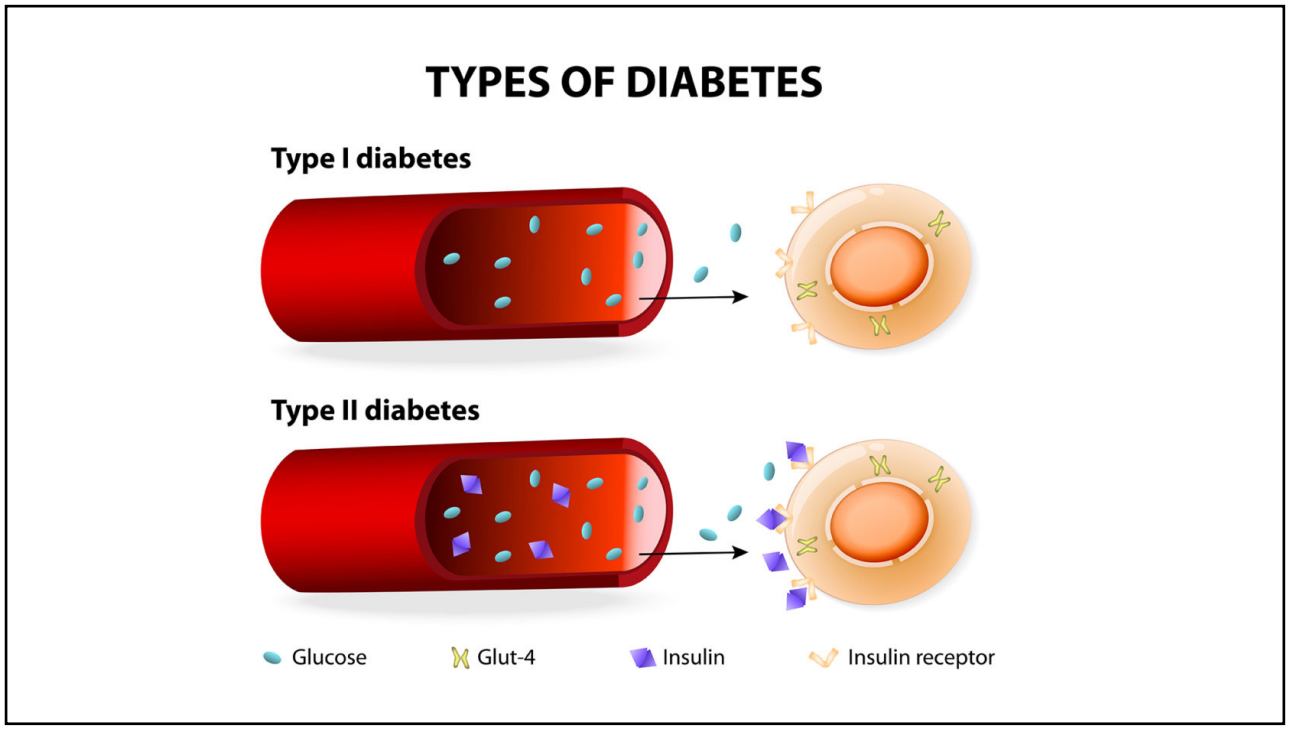

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Type 1 diabetes mellitus

- Type 1 diabetes accounts for about 15% of all diabetic patients.
- It can occur at any age but is most common in the young, with a peak incidence between 9 and 14 years of age.
- The complete lack of insulin is a consequence of the autoimmune destruction of insulin producing beta cells.
- There may be an environmental precipitating factor such as a viral infection.
- The presence of islet cell antibodies in serum predicts future development of diabetes.

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Type 2 diabetes mellitus

- Type 2 diabetes accounts for about 85% of all diabetic patients and can occur at any age.
- It is most common between 40 and 80 years but is now being reported in adolescent and even pediatric populations.
- In this condition there is resistance of peripheral tissues to the actions of insulin, so that the insulin level may be normal or even high.
- Obesity is the most commonly associated clinical feature.

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Other types of Diabetes mellitus

- ***1. Gestational diabetes***: develops in some women during the late stages of pregnancy.
- It is caused by the hormones of pregnancy or by a shortage of insulin.
- ***2. Pre-diabetes, in pre-diabetes***, blood glucose levels are higher than normal but not high enough to be characterized as diabetes.
- However, many people with pre-diabetes develop type 2 diabetes within 10 years.
- Pre-diabetes also increases the risk of heart disease and stroke.
- With modest weight loss and moderate physical activity, people with pre-diabetes can delay or prevent type 2 diabetes.

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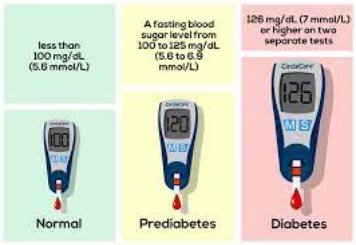
Diagnosis of Diabetes measurements

- The following tests are used for diagnosis:-
  - ***A fasting plasma glucose test FBS*** measures the blood glucose after at least 8 hours of fasting. This test is used to detect diabetes or pre-diabetes.
  - ***A post meal plasma glucose test*** measures the blood glucose after usual adequate meals. Patient must complete meal within 15-20 min. Specimen is collected at 2 hr from beginning of meal.
  - ***An oral glucose tolerance test*** measures the blood glucose after at least 8 hours of fasting and 2 hours after drinking a glucose- containing beverage. This test can be used to diagnose diabetes or pre-diabetes.
  - **In *a random plasma glucose test RBS***, blood glucose can be checked without regard to when one ate the last meal. This test, along with an assessment of symptoms, is used to diagnose diabetes but not pre-diabetes.



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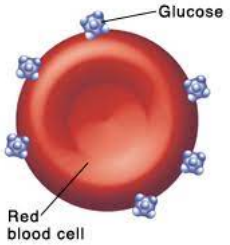
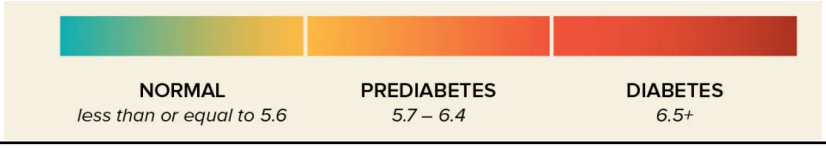
BS and HbA1c



- There are two measurements are widely per-formed:

1. A direct measurement of glucose itself. FBS and RBS.
2. A glycated hemoglobin HbA1c:

- The formation of the sugar-hemoglobin linkage indicates the presence of excessive sugar in the bloodstream, often indicative of diabetes.
- A1C is of particular interest because it is easy to detect. The process by which sugars attach to hemoglobin is called glycation.



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HbA1c, HgbA1c, Hb1c, etc., also A1C

- Glycated hemoglobin (glycohemoglobin, hemoglobin A1c, HbA1c, less commonly HbA1c, HgbA1c, Hb1c, etc., also A1C informally with patients)
- It is a form of hemoglobin (Hb) that is chemically linked to a sugar. Most monosaccharides, including glucose, galactose and fructose spontaneously (i.e. non-enzymatically) bond with hemoglobin, when present in the bloodstream of humans.
- However, glucose is less likely to do so than galactose and fructose (13% that of fructose and 21% that of galactose), which may explain why glucose is used as the primary metabolic fuel in humans.
- The formation of the sugar-hemoglobin linkage indicates the presence of excessive sugar in the bloodstream, often indicative of diabetes.

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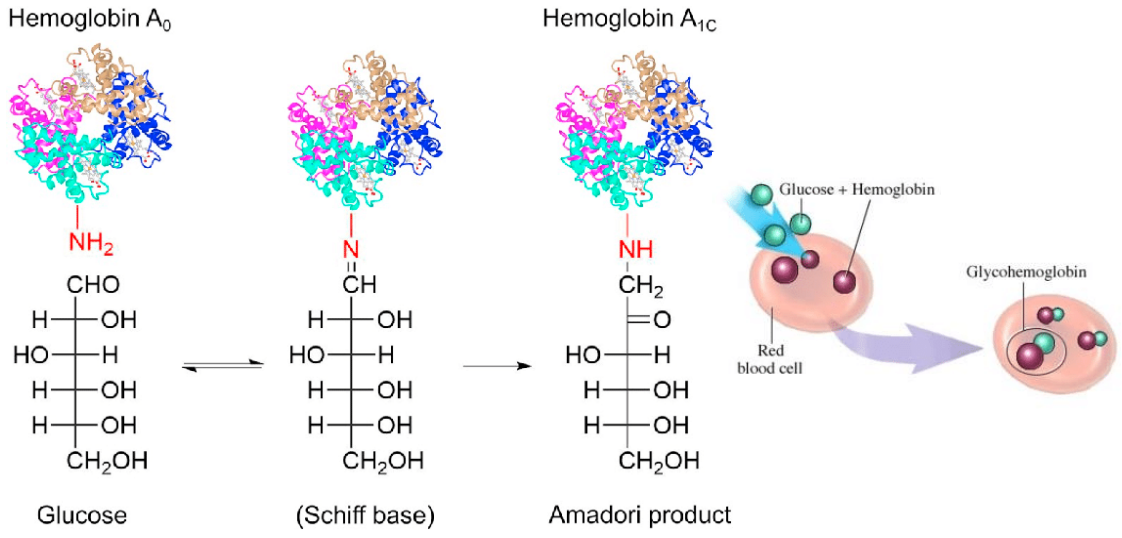
Reaction of glucose with HB



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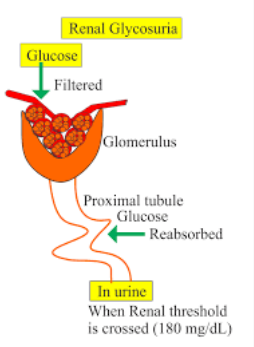
Glucose in urine

- **Glycosuria**
- Glycosuria is a term that defines the presence of reducing sugars in the urine, such as glucose, galactose, lactose, fructose, etc.
- The presence or absence of glycosuria has no role in the screening or the diagnosis of diabetes.
- However, the false-negative rate for this test is unacceptably high.



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Pros and Cons

Table 32.2 **Pros and cons of using measurement of glycated haemoglobin (HbA_{1c}) to diagnose diabetes mellitus**

Pros	Cons
Patient does not have to fast	HbA_{1c} has comparatively poor sensitivity in the diagnosis of diabetes
HbA_{1c} is more closely associated with chronic complications of diabetes than fasting glucose	HbA_{1c} not as closely associated with cardiovascular disease as post-prandial glucose measurements, e.g. 2-hour glucose
Pre-analytic stability of HbA_{1c} superior to glucose	HbA_{1c} is unreliable in conditions where red cell turnover is increased e.g. chronic anaemia, haemolysis, haemoglobinopathies
Using the same marker to diagnose and monitor more 'seamless'	Standardization of HbA_{1c} measurement is not as good as glucose

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Ketones in urine or blood

- **Ketones in urine or blood**
- ketone bodies are formed in the urine and blood when the body break down fat too fast.
- This is a serious condition called ketoacidosis.
- The term 'ketone bodies' refers to acetone and the keto-acids acetoacetate and β-hydroxybutyrate.
- These are frequently found in uncontrolled diabetes (diabetic ketoacidosis).
- They are also found in normal subjects as a result of starvation or fasting, and sometimes in alcoholic patients with poor dietary intake (alcoholic ketoacidosis).

Urine Ketone Levels	0.1 - 0.2 mmol/L	0.3 - 0.6 mmol/L	0.7 - 5 mmol/L	5 – 8 mmol/L	9 – 15 mmol/L
What This Means	High-carb diet, low ketone levels	Beginning nutritional Ketosis	Nutritional Ketosis	Medically therapeutic Ketosis	Starvation Ketosis

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Methodology for glucose mesurements

- Glucose oxidase method:
- Glucose oxidase (GOD) oxidizes glucose to gluconic acid. Hydrogen peroxide is produced in this reaction. In presence of peroxide hydrogen peroxidase (POD) reacts with 4-aminoantipyrine and phenol to form red coloured quinoneimine dye. The intensity of colour is directly proportional to the glucose concentration. This method is highly specific for glucose and does not involve any other sugar.

$$\text{Glucose} + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{phenol} + \text{4-aminoantipyrine} \xrightarrow{\text{POD}} \text{Quinoneimine dye}$$

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Abnormalities of lipid metabolism

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1

What are lipids? Classification?

- **Lipids** are heterogeneous group of oily/greasy organic compounds, which are relatively insoluble in water but soluble in organic solvents. It involves diverse group of compounds.
- **CLASSIFICATION**
- Simple Lipids
- Compound Lipids, and
- Derived Lipids.

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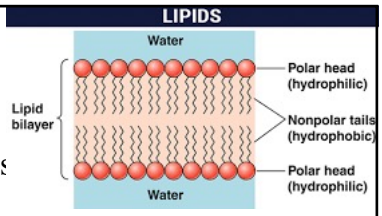
Simple Lipids

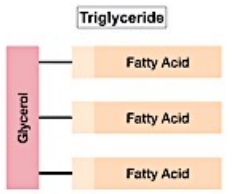


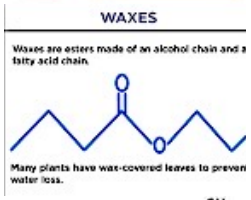
- These lipids are esters of fatty acids with certain alcohols
- These are sub classified according to the nature of alcohol.
- Fatty acids: It consists of long chain of hydrocarbon having carbonyl group at one end and methyl group at another end.
- Fatty acids differ in chain length and degree of unsaturation.
- These are further classified as saturated fatty acids (without double bond in carbon chain) and unsaturated fatty acids (with double bond in carbon chain).

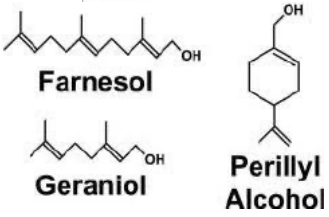
3

Saturated and unsaturated lipids?

- In addition to straight chain compounds a number of branched chain and cyclic fatty acid, both, saturated and unsaturated are found.
- **Triglycerides**: These are neutral esters of glycerol and fatty acids, e.g triscarin
- **Waxes**: Waxes are esters or fatty acids with monohydric alcohol, e.g. spermaceti
- **Isoprenoid lipids**: These are chemically unrelated to fats and phospholipids but their solubility are same.







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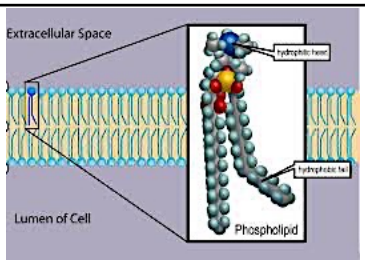
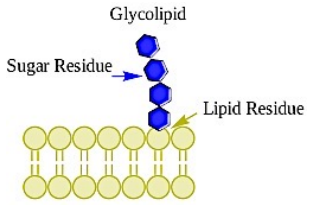
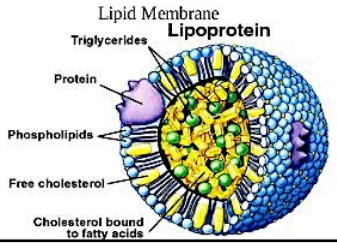
Compound Lipids

- These are esters of fatty acids with alcohols, containing additional groups. These are sub classified as—
- **Phospholipids**: These lipids contain phosphorus as additional group, e.g. phosmalogen
- **Glycolipid**: Combination of carbohydrate and lipids are glycolipids. These are found in chloroplast membranes.
- **Lipoproteins**: Combination of lipid and protein are lipoprotein, e.g. cholestrol, glycerol







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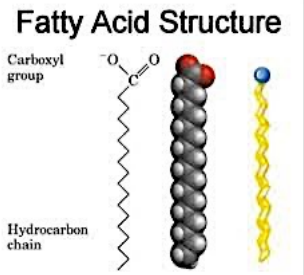
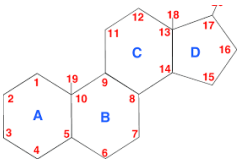
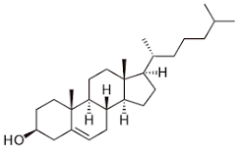
Derived Lipids

- These are the lipids, which arise as a result of partial hydrolysis of lipid.
- **Fatty acids**: These are hydrolysis products of fats and other lipids.
- Naturally occurring fats generally contain an even number of carbon atoms. These may be saturated or unsaturated, e.g. linoleic acid
- **Steroids**: Steroids are naturally occurring cyclic compounds (**17 carbon atoms connected with 4 fused rings in a specific way**), also called corticosteroids. These have different physiological properties, e.g. testosterone.
- Cholesterol is an important **Steroids (sterols).**



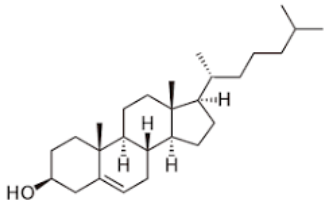




6

Where cholesterol comes from?

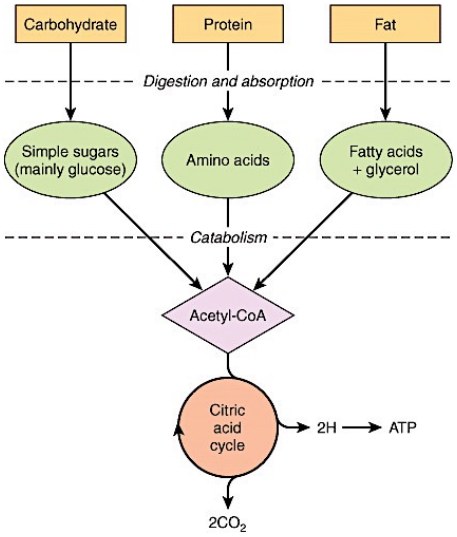
- The cholesterol in your blood comes from two sources: **the foods we eat and secondly the liver**. liver makes all the cholesterol that body needs.
- Cholesterol is the precursor of steroid hormones, D vitamins, bile acids/salts, and is a key component of biological membranes.
- Dietary cholesterol is absorbed mainly in the duodenum and jejunum of the gastrointestinal tract (GIT). Absorption depends on solubilization with other fats and bile salts to form minute droplets called mixed **micelles**
- When cholesterol becomes high?



7

Energy from Lipid Metabolism

- About 40 percent of the bodies caloric intake is derived from lipids and almost all of these calories come from fats, the triglycerols.
- The use of fatty acids metabolism is predominantly performed in liver.
- The liver may play a modifying part in fat storage and retrieval.
- The major source of lipids entering the liver in free fatty acid in the systemic blood plasma complexed with albumin.



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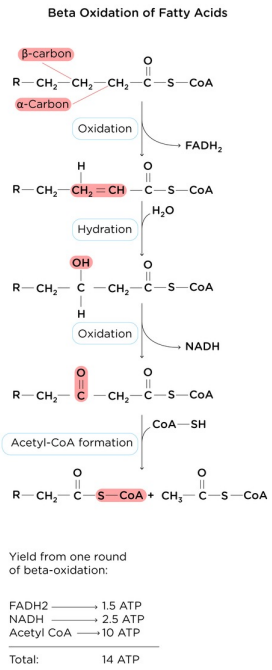
Oxidation of Fatty acid

- Fatty acid oxidation yields twice the usable chemical energy that carbohydrates can deliver.
- As an example, 130 mols of ATP result from the oxidation of one mol of palmitic acid, as compared to 38 mols of ATP from one mol of glucose.
- On a weight basis, the caloric yield from fatty acids is about double that from carbohydrates; 9 kcal/g from fat vs. 4kcal/g from carbohydrate or protein).
- The major aspects of lipid metabolism are involved with Fatty Acid Oxidation to produce energy or the synthesis of lipids, which is called **Lipogenesis**.



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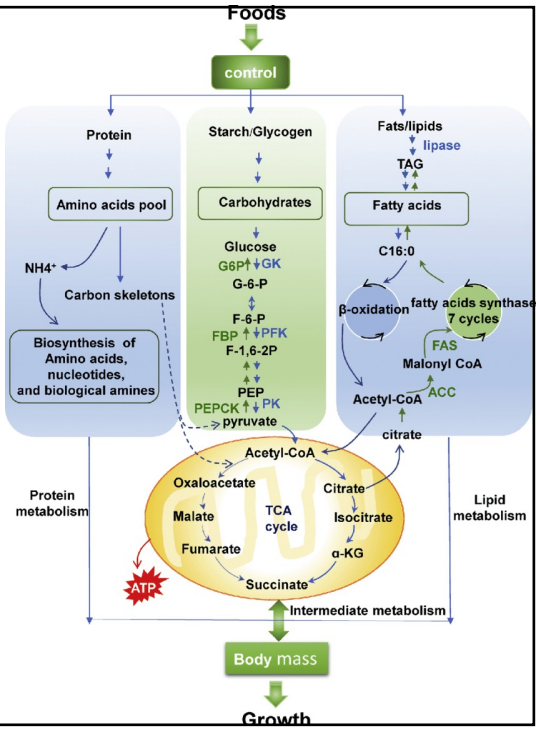
Biosynthesis & metabolism of lipids?

- Biosynthesis of fatty acids requires acetyl-Co-A as a key intermediate.
- The first step in lipid metabolism is the hydrolysis of the lipid in the cytoplasm to produce glycerol and fatty acids.
- Free fatty acid enter mitochondria and undergo a process called β-oxidation for degradation to acetyl-Co-A.



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Diseases related to Lipid Metabolism

- **Steatorrhoea**: is **an increase in fat excretion in the stools**. Steatorrhea is one of the clinical features of fat malabsorption.
- **Obesity** is another disorder due to accumulation of excess of body fat.
- **Lipidosis**: This means the abnormal lipoproteins in blood or specific lipids in tissues.
- **Hyperlipidemia:** This is a condition in which plasma cholesterol or plasma triglyceride level is increased. This condition occurs due to inherent genetic defects.

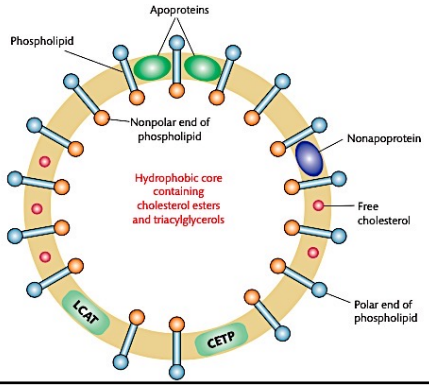
11

What are Lipoproteins? Lipids + Proteins

- lipids can associate with proteins called **apoproteins** to form water soluble complexes or **lipoproteins** if they are to circulate.
- **Classification of lipoproteins and lipoprotein particles**
- Lipoprotein particles are mainly classified according to their density, giving:

1. Chylomicrons.
2. very low density lipoproteins. VLDL
3. low density lipoproteins. LDL
4. high density lipoproteins HDL

	Diameter (nm)	Density (g/L)
Chylomicrons	80–500	<950
VLDL	30–80	950–1006
IDL	25–30	1006–1019
LDL	19–25	1019–<1063
Lp(a)	0–25	1052–<1063
HDL_2	8–11	1063–<1125
HDL_3	6–9	1125–1210



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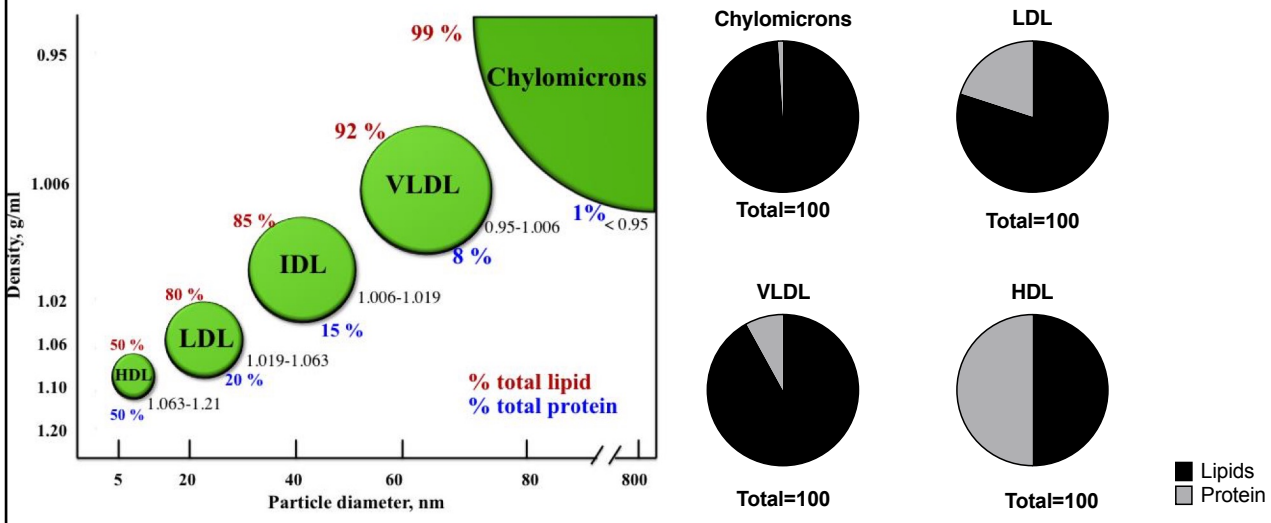
How much protein and lipid are in each?



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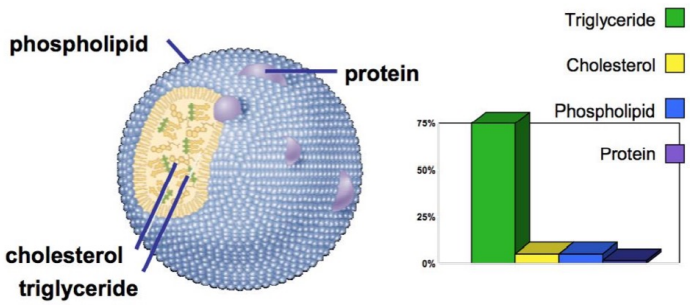
Chylomicrons

- Chylomicrons are involved in the transport of dietary lipids.
- Chylomicrons are **large triglyceride-rich lipoproteins produced in enterocytes from dietary lipids**—namely, fatty acids, and cholesterol.
- Chylomicrons are composed of a main central lipid core which largely transport dietary triacylglycerols from GIT (Gastrointestinal tract) to peripheral tissues



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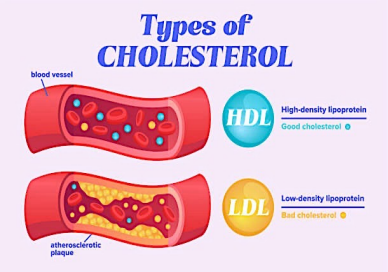
LDL low density lipoproteins

- sometimes called “bad” cholesterol, **makes up most of the body's cholesterol**.
- High levels of LDL cholesterol raise the risk for heart disease and stroke.
- Delivers cholesterol to cells in the body by binding to LDL receptors
- Most of the cholesterol in the blood is associated with LDL.
- There is a correlation between increasing concentrations of cholesterol in samples of serum and the risk of the patient developing **coronary heart disease.**
- Optimal: **Less than 100 mg/dL**



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HDL, *High density lipoproteins*

- *High density lipoproteins* is known as the "good" cholesterol because it helps remove other forms of cholesterol from the bloodstream.
- The main structural protein in HDL is apoprotein A1, which is synthesized in the liver and intestine.
- Typically, there are four to seven molecules of apoA1 per HDL particle.
- It absorbs cholesterol and carries it back to the liver.

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VLDL very low density lipoproteins

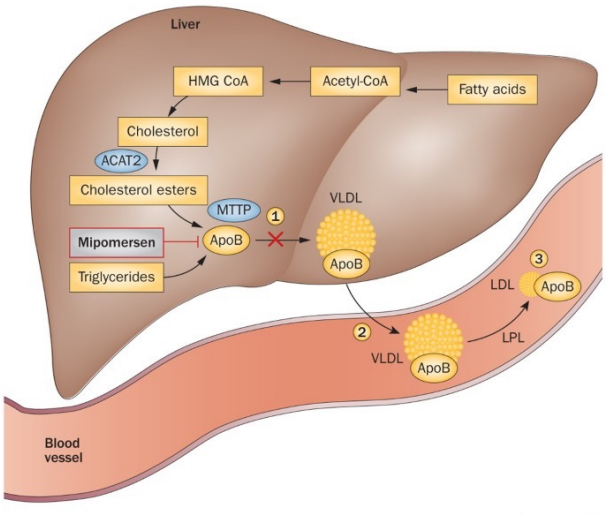
- **VLDL stands for very-low-density lipoprotein**.
- The liver makes VLDL and releases it into your bloodstream.
- The VLDL particles mainly carry triglycerides, another type of fat, to the tissues.
- VLDL is similar to LDL cholesterol, but LDL mainly carries cholesterol to the tissues instead of triglycerides.
- Normal VLDL cholesterol level is **between 2 and 30 mg/dL**.

$$\text{LDL cholesterol} = \text{total cholesterol} - \text{HDL cholesterol} - \frac{\text{TAG}}{2.2}$$

17

Hypercholesterolaemia

- Hypercholesterolaemia is defined as high concentrations of cholesterol in blood that increase the risk of **cardiovascular disease**.
- It is recommended that total cholesterol concentration should be less than 5.0 mmol/L and that LDL cholesterol should be less than 3.0 mmol/L in healthy people.



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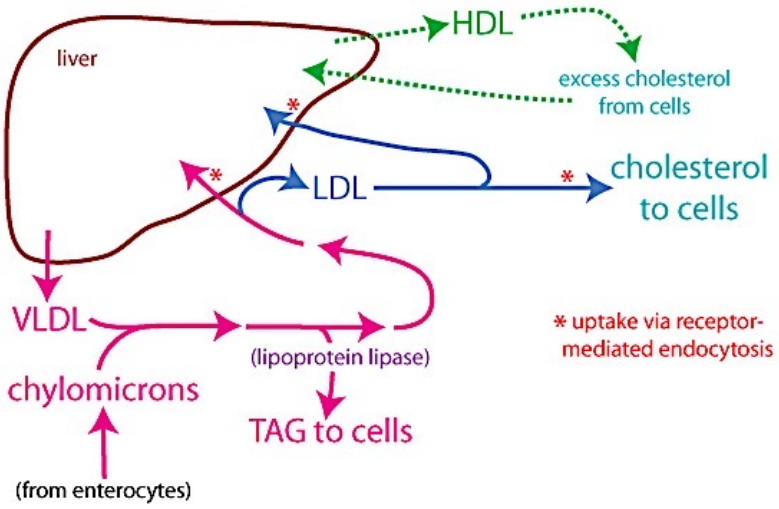
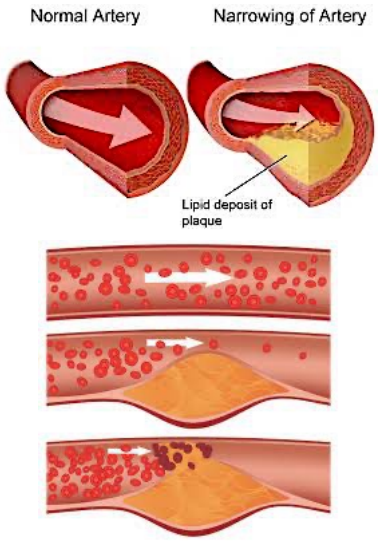
Metabolism of lipoproteins



19

lipoprotein disorders

- Lipoprotein disorders are some of the commonest metabolic diseases seen in clinical practice.
- They may present with their various sequelae which include:
- Coronary heart disease (CHD).
- Acute pancreatitis.
- Failure to thrive and weakness.



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Clinical disorders of lipid metabolism

- The Fredrickson classification is still commonly used to classify hyperlipoproteinaemias by phenotype.
- The genetic and environmental nature of many causes of primary hyperlipidaemia are, as yet, unknown.
- Secondary causes of hyperlipidaemia are common and include hypothyroidism, diabetes mellitus, liver disease and alcohol abuse.

21

Liver function tests
Bilirubin

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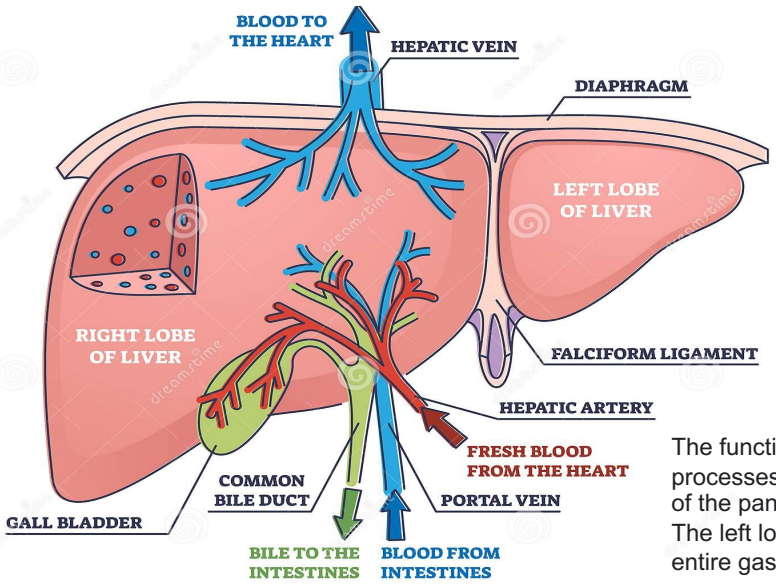
1

The role of liver?

- The liver has the three main roles:
- Detoxification: the liver takes compounds which are harmful and not very soluble and makes them less harmful and more soluble (so easier to store or excrete) this is mainly done with enzymes.
- Storage: in the liver, for example, stores glycogen which is the stored form of glucose.
- Production the liver also produces some of the chemicals such as C-reactive protein fiber on a and transfer in

2

A general anatomy of liver



The functional right lobe of the liver processes blood coming from the head of the pancreas and others.
The left lobe receives blood from the entire gastrointestinal tract.

3

Liver function tests LFTs

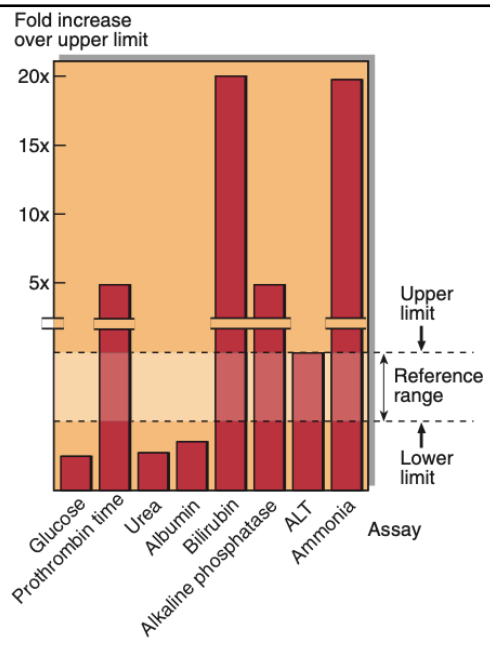
- LFTs are measurements of blood components that simply provide a ability to get rid of toxins, and the type of liver damage.
- They do not assess quantitatively the capacity of the liver to carry out the functions described above.
- These biochemical investigations can assist in differentiating the following:

1. Block the biliary tract.
2. acute hepatocellular damage.
3. chronic liver disease.

4

Liver function test includes?

- Serum bilirubin
- SGPT
- SGOT
- Serum protein, serum albumin concentration.
- Alkaline phosphatase
- Urine — bile salts, bile pigments and urobilinogen



Laboratory findings in hepatic failure.

5

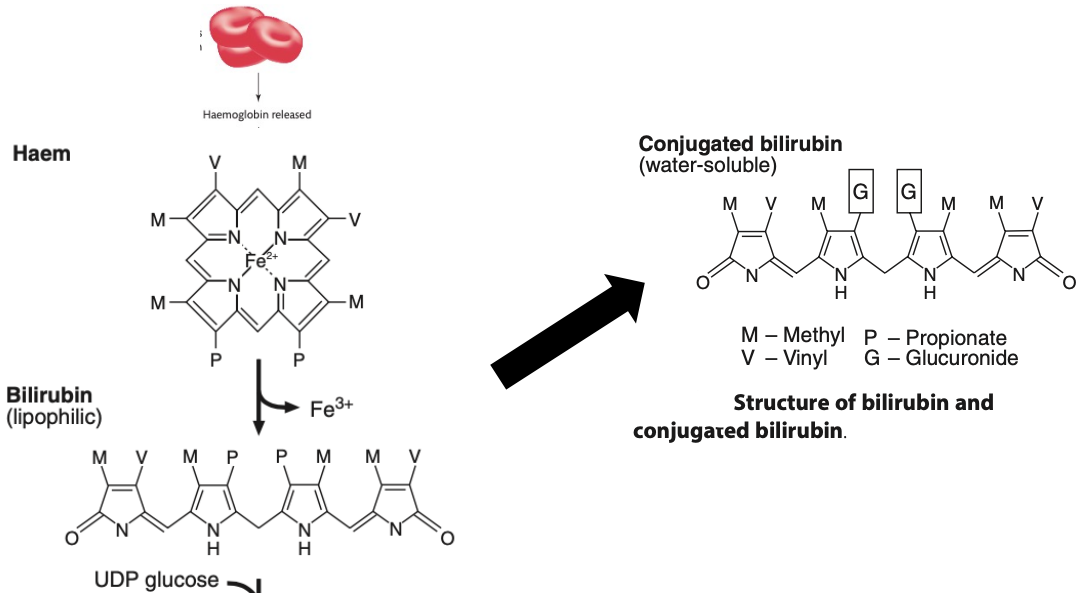
Estimation of Serum Bilirubin

- Bilirubin originates from the break down of hemoglobin.
- It is a waste product, and the body eliminates this compound through the bile (from liver) into the intestine and ultimately through the stool.
- Only a small fraction of bilirubin metabolite is recycled through the body and a part of it is excreted through the urine.
- Serum Bilirubin can be present in two forms:

1. Conjugate bilirubin:
2. Unconjugated bilirubin:

6

Structure of bilirubin and conjugated bilirubin



Structure of bilirubin and conjugated bilirubin.

7

Conjugate and Unconjugated bilirubin

1. **Conjugate bilirubin**. It is conjugated with **glucuronic acid** to form bilirubin glucuronide. It is water-soluble. It reacts directly in aqueous solution without alcohol. Therefore, it is also called as direct bilirubin.
2. **Unconjugated bilirubin:** It is a free bilirubin formed from proto-porphyrin component of heme. It is insoluble in water and carried away to liver by serum albumin. It reacts indirectly with presence of alcohol. Therefore, it is also called as indirect bilirubin.

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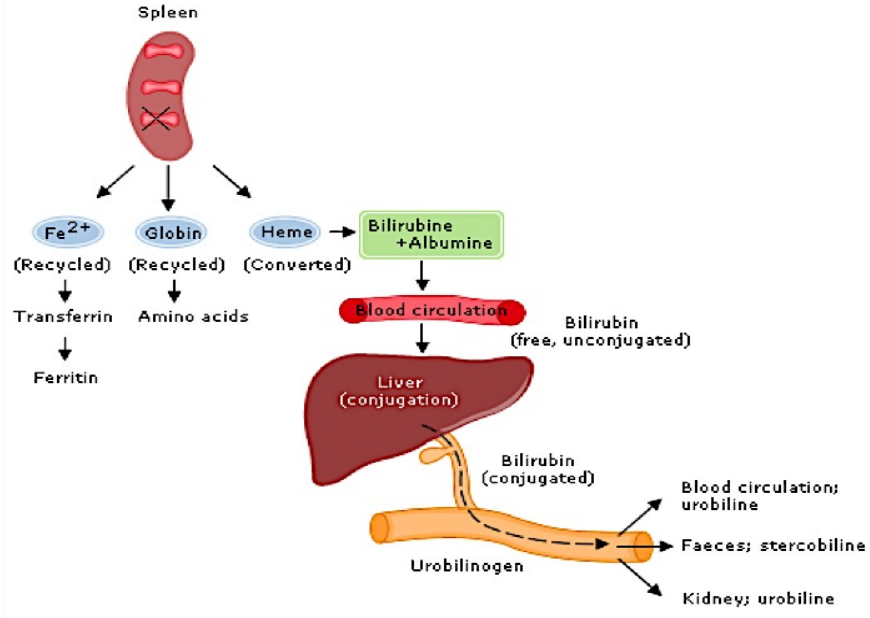
Where the bilirubin comes from?



9

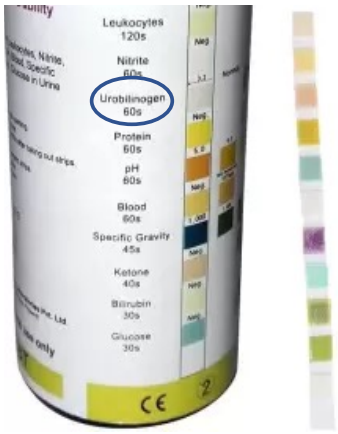
More about Bilirubin

- **Bilirubin**
- An adult normally produces about 450 µmol of bilirubin daily.
- The conjugated bilirubin is excreted into the bile.
- Normal bile contains bilirubin mono-glucuronide as 25% and the glucuronide as 75% of the total, accompanied by traces of unconjugated bilirubin.
- The main functional components of the bile are the bile salts, which are involved in fat digestion and absorption from the small intestine.
- In the terminal intestine and colon, the bilirubin conjugates are attacked by bacteria, most of which are excreted in faces.

10

When patient becomes jaundiced?

- Small amounts of these tetra-pyrroles are found in urine in which they are known as ***urobilinogen***.
- When the biliary tract becomes blocked, bilirubin is not excreted, and serum concentrations rise.
- The patient becomes jaundiced.
- The jaundiced patient is described further on pages 58–59.



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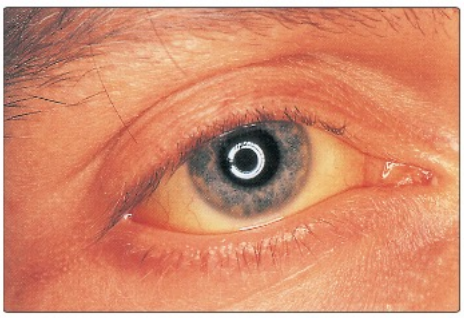
Jaundice!

- Jaundice is a yellow discoloration of the skin or sclera (see Fig).
- This is due to the presence of bilirubin in the plasma and is not usually detectable until the concentration is greater than about 50 μmol/L.
- Normally the bilirubin concentration in plasma is less than 21 μmol/L.



Jaundice in the sclera of an eye.

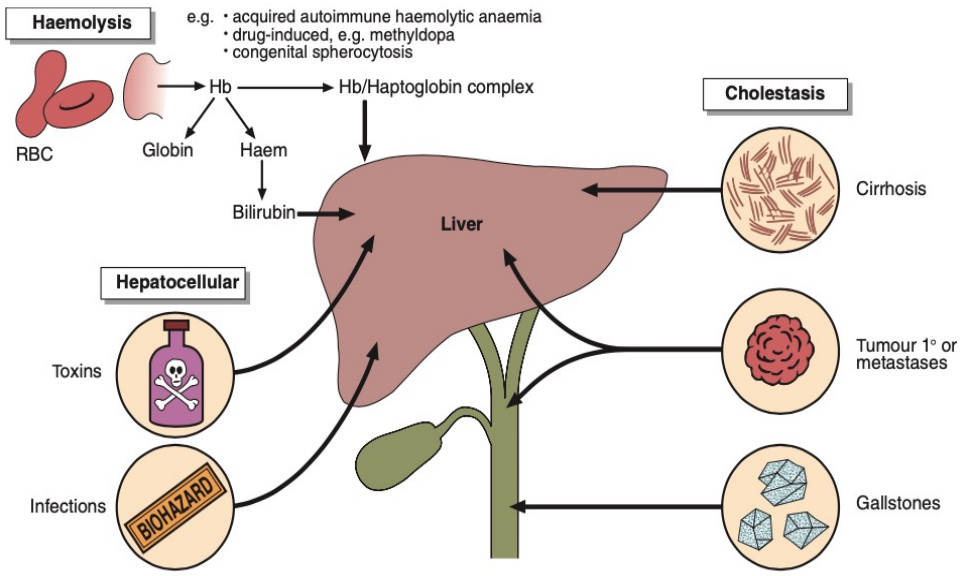
12

Why bilirubin levels rise?

- There are three main reasons why bilirubin levels in the blood may rise (see Fig).
- ***Hemolysis***: The increased hemoglobin breakdown produces bilirubin, which overloads the conjugating mechanism.
- ***Failure of the conjugating mechanism within the hepatocyte***.
- ***Block in the biliary duct.***
- ***Inherited disorders of bilirubin metabolism e.g Gilbert's disease***

13

Causes of jaundice



14

1-Hemolysis or Haemolysis

- Cholestasis is defined as stagnation (the state of not flowing or moving), or at least a marked reduction, in bile secretion and flow
- Increased bilirubin production caused by hemolysis gives an unconjugated ***hyperbilirubinemia***. This is associated liver function in babies. A rapidly rising bilirubin in a newborn should be carefully monitored as it may give rise to brain damage (kernicterus).
- If the concentration approaches 200 µmol/L, phototherapy should be used to break down the molecule in the skin and reduce the level. If the concentration rises above 300 µmol/ L, exchange transfusion may be necessary.

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Dignosis as a biochemists

Table 29.1 **Laboratory differential diagnosis of jaundice**

	Haemolytic	Cholestatic	Hepatocellular
Features	■ Bilirubin concentration variable ■ No bilirubin in urine ■ Reticulocytosis ■ Haemoglobin ↓ ■ Haptoglobin ↓ ■ LDH may ↑	■ Bilirubin may be ↑↑↑ ■ Bilirubin in urine. ■ Alkaline phosphatase, usually >3× upper limit of reference range ■ AST, ALT + LDH usually modestly ↑	■ AST + ALT ↑↑ ■ Bilirubin ↑ later ■ Bilirubin in urine ■ Alkaline phosphatase ↑ later

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2-Extrahepatic biliary block

- Gallstones can partially or fully block the bile duct.
- Such a blockage is known as extrahepatic obstruction.
- If the blockage is complete, both bilirubin and alkaline phosphatase are raised.
- There will be a little or no urobilinogen in urine.
- Stools will be pale in color.

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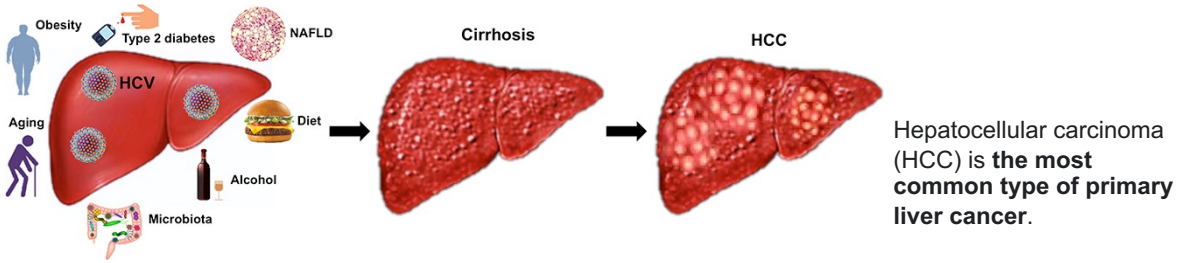
3-Hepatocellular damage

- Dray to damage to the hepatocytes by infection or toxins, rather than damage to the biliary tract.
- The most common causes of acute jaundice seen in adults are viral hepatitis and paracetamol poisoning.
- In these cases, not only are the bilirubin and alkaline phosphatase levels raised, but AST and ALT are elevated indicating hepatocellular damage.



Hepatocellular carcinoma (HCC) is **the most common type of primary liver cancer**.

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If levels rise too high!

- Since Unconjugated bilirubin is not water-soluble, it binds to albumin from which it may be transferred to other proteins such as those in cell membranes.
- It is neurotoxic, and if levels rise too high in newborn, permanent brain damage can occur.
- This combination of pale stools and dark urine is characteristic of extrahepatic block of the biliary tract.
- This needs surgery!

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Conclusion

- Jaundice indicates that there is an elevated concentration of bilirubin in plasma.
- In newborn, it is important to determine the concentration of total and conjugated bilirubin to aid diagnosis and decide on treatment.
- n adults, the most common cause of jaundice is obstruction, and this is confirmed by the elevation of both bilirubin and alkaline phosphates.

20

What we do mesure in lab?

- Direct and indirect bilirubin
- High levels of indirect Bilirubin would indicate a liver problem or a pre- haptic block.
- High levels of direct would indicate that the liver is able to convert the bilirubin but there could be a post hypnotic blockage.
- Total Bilirubin.
- Direct (also called conjugated) bilirubin: less than 0.3 mg/dL (less than 5.1 μmol/L) Total bilirubin: 0.1 to 1.2 mg/dL (1.71 to 20.5 μmol/L)

21

Liver function tests liver enzymes

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1

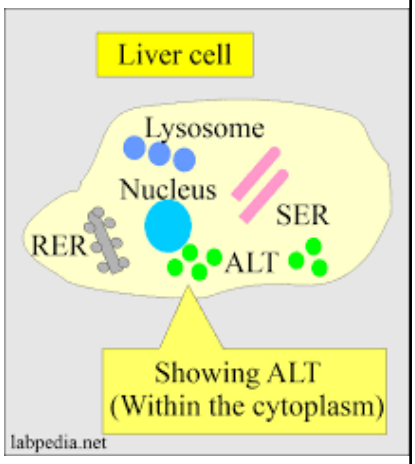
Liver function test includes?

- Serum bilirubin (done last lecture).
- SGPT
- SGOT
- Serum protein, serum albumin concentration.
- Alkaline phosphatase
- Urine — bile salts, bile pigments and urobilinogen

2

What are The aminotransferases (AST and ALT)?

- The activities of two aminotransferases, AST and ALT, are widely used in clinical practice as a sensitive, although non-specific, indicating of acute damage to hepatocytes irrespective of its etiology.
- Liver damage include hepatitis and drug overdose.
- Acute liver damage due to shock, severe hypoxia and acute cardiac failure is also seen.



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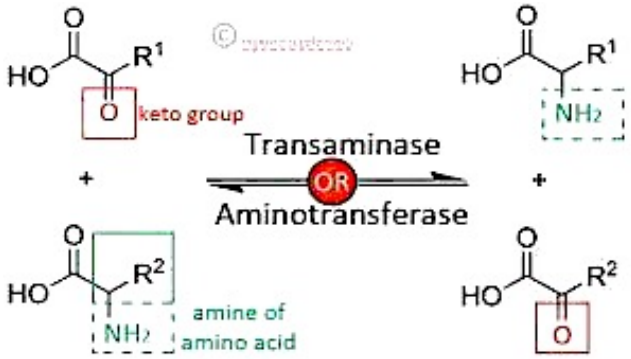
Transaminases?

- Transamination is a process in which an amino group is transferred from an amino acid to an alpha keto acid. It is an important step in amino acid metabolism.
- The enzymes responsible for transamination are called Transaminases.



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How?

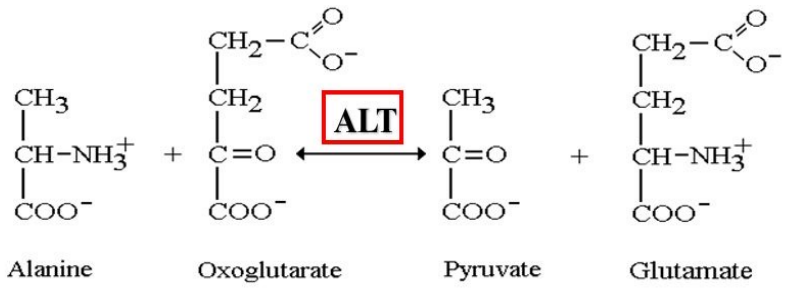
- Two useful transaminases are Glutamate oxaloacetate transaminase or GOT (also called as asparate aminotransferase or AST) and Glutamate pyruvate transaminase or GPT (also called as alanine aminotransferase or ALT).

L-asparate + Oxoglutarate $\xrightarrow{\text{GOT/AST}}$ Oxaloacetate + Glutamate

L-alanine + Oxoglutarate $\xrightarrow{\text{GPT/ALT}}$ Pyruvate + Glutamate

5

GPT

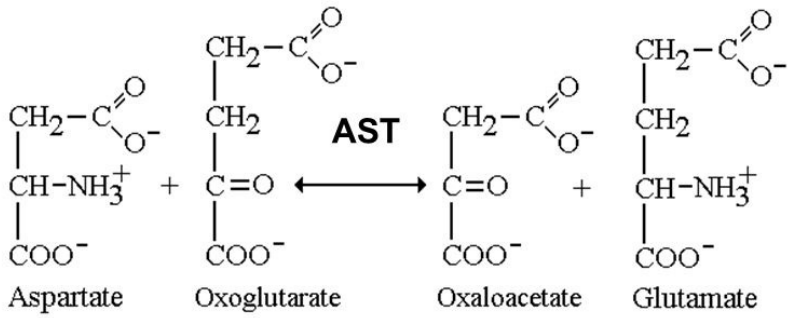


- **ALT or sGPT (serum glutamate pyruvate transaminase)**

GPT (ALT) catalyses the transfer of amino-groups from alanine to 2-oxoglutarate and thus the formation of glutamate and pyruvate.

6

GOT



- **AST or sGOT (serum glutamate oxaloacetate transaminase)**

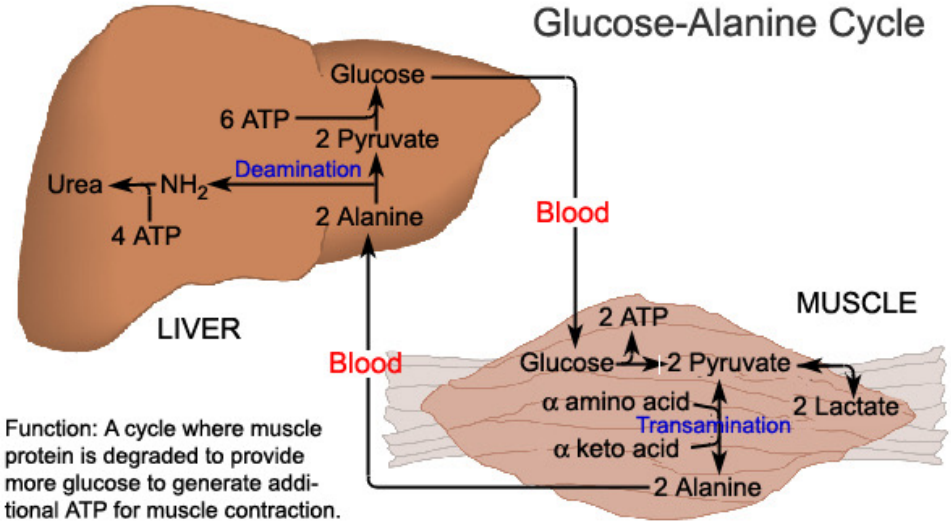
7

Where do GOT & GPT found?

- Aspartate aminotransferase is found in liver, heart, skeletal muscle, kidney, brain, and red blood cells, and whilst ALT has a similar distribution, its concentrations are lower in extrahepatic tissues.
- Aspartate aminotransferase is a part of the malate-aspartate shuttle in the myocardium, is involved in gluconeogenesis in the liver and kidney.

8

Liver and muscle



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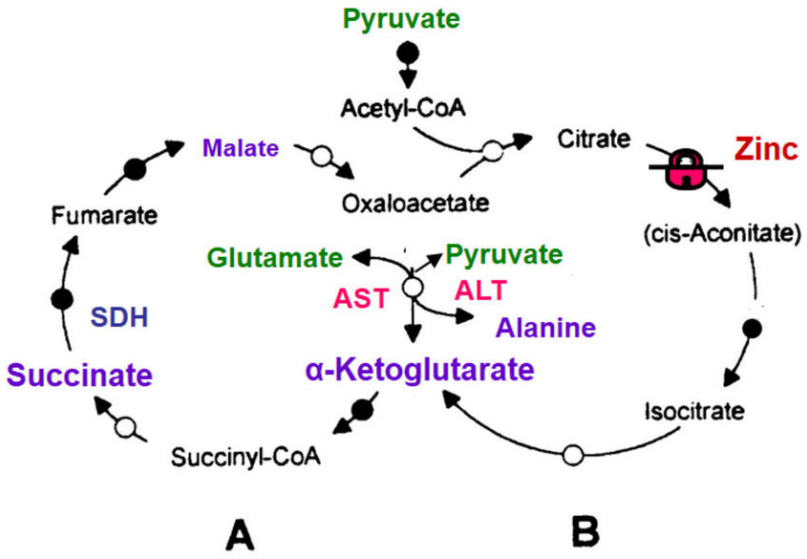
The Tricarboxylic Acid Cycle in the presence of pyruvate and glutamate operates as two coupled cycles A and B



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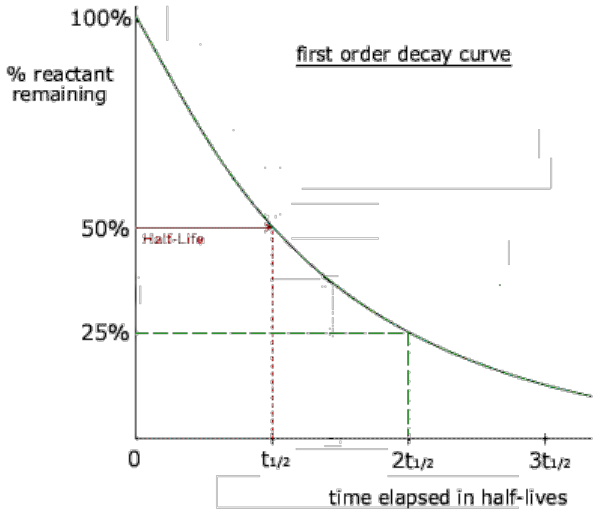
Pathological of Aminotransferases

- The two most widely measured for clinical purposes are aspartate aminotransferase (AST) and alanine aminotransferase (ALT).
- They have no functional significance in blood but are indicators of hepatic damage.
- Their activities are typically 3,000–7,000-fold higher in hepatocytes than in plasma.
- Although dependent on the methodology used for their measurement typical reference ranges for AST and ALT are 5–45 IU/L.

11

Half life?



- Aspartate transaminase exists in both cytosolic and mitochondrial forms (half-life of cytosolic AST is 47 h
- whereas that for mitochondrial AST is 87 h), but ALT is in a cytosolic form only (half-life of 17 h).

12

How could alcohol misuse affect AST/ALT ratio

- Consideration of the results of the aminotransferases as a ratio can be helpful as an AST/ALT ratio greater than two is suggestive of alcohol misuse because of release of mitochondrial AST due to damage from alcohol metabolites.
- The higher the relative increase in AST compared to alkaline phosphatase (ALP) the more likely it is that the patient has hepatitis.

13

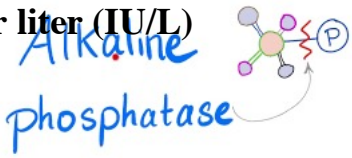
Do AST & ALT change in the day and night?

- Intra-individual variation in the aminotransferases is low, with a day-to-day variation in AST of approximately 5–10% and for ALT 10–30%.
- There is no *diurnal rhythm* for AST, but ALT activity is up to 45% lower at night compared to a peak in the afternoon.

14

Alkaline Phosphatase

- Alkaline Phosphatase is present in most tissues but is present in high concentration in liver, bones, intestines, spleen, placenta and kidney.
- It is involved in transport of phosphate across cell membrane.
- It has hydrolytic and phosphate transferase activity.
- The normal range is 44 to 147 international units **per liter (IU/L) or** 0.73 to 2.45 microkatal per liter (μkat/L).



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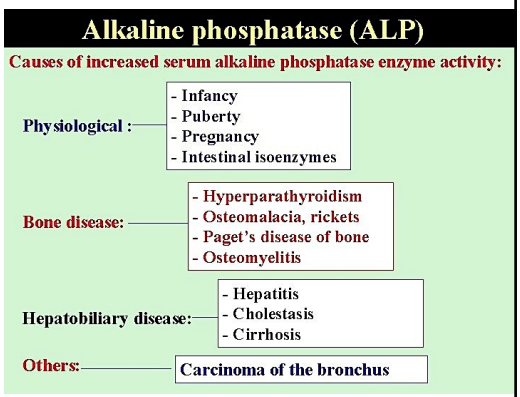
How could ALP be increased ?

- Increases in alkaline phosphatase activity in liver disease are the result of increased synthesis of the enzyme by cells lining the bile canaliculi, usually in response to cholestasis, which may be either intra- or extrahepatic.
- It also occurs in cirrhosis.
- Liver is not the sole source of alkaline phosphatase activity.
- Substantial amounts are present in bone, small intestine, placenta and kidney.
- In normal blood, the alkaline phosphatase activity is derived mainly from bone and liver, with small amounts from intestine.



16

Liver function tests
Serum Proteins examinations

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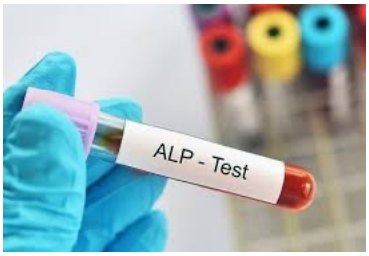
Department Of Biophysics

1

Serum Proteins examinations



- Serum proteins constitute of **albumin and globulin.**
- Its detection is useful in diagnosis of liver diseases.
- **Clinical Significance:**
- Determination of serum total proteins is useful in screening for nutritional deficiencies and gammopathies.
- It is increased in multiple liver diseases such as myeloma (**cancer of the plasma cells)**.
- Decreased protein synthesis like in case of severe liver disease, increased protein loss like in severe skin disease, GI disease, Renal disease and blood loss. Increased catabolism like in case of fever or inflammation, malignancy etc.

2

Specific protein markers? In plasma

- The plasma of blood is rich in numerous proteins that perform a variety of different functions.
- The proteins present in plasma include a number of enzymes, transport proteins, protein hormones, cytokines, clotting factors, and complement proteins.
- The liver is the principal site of the synthesis of all plasma proteins
- These proteins are sometimes called specific proteins.
- We can examine the various proteins in plasma and the value of measuring their concentrations in relation to disease investigation.
- Specific proteins comprise approximately 80% by weight of the 70 g/L of total protein in plasma.

3

Specific proteins

The major proteins in the plasma.

Protein	Normal concentration in serum (g/L)			Response to an acute phase reaction
	Mean	Lower limit	Upper limit	
Total protein	70	63	83	No effect
Albumin	40	32	48	Decrease
α_1-antitrypsin	1.5	0.9	1.8	Increase
α_1-acid glycoprotein (orosomucoid)	1.0	0.4	1	Increase
Haptoglobin	1.5	0.5	2.6	Increase
Caeruloplasmin	0.3	0.2	0.6	Increase
Transferrin	2.5	1.9	3.5	Decrease
Complement C3	1.0	0.8	2.14	Increase
Complement C4	0.3	0.13	0.6	Increase
IgG	10	6	13	No effect
IgA	2.0	0.8	3.7	No effect
IgM	1.5	0.4	2.2	No effect

4

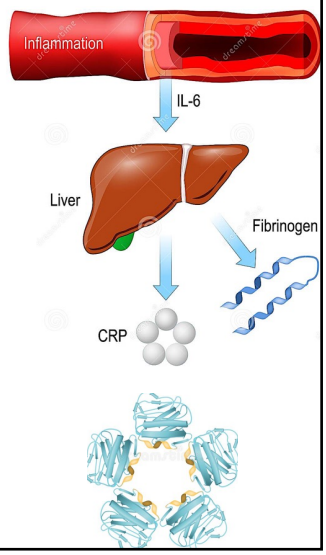
The major and the manor cause of protein deficiency

- The major cause of a change in the rate of synthesis of proteins is the **acute phase response**, which is a rapid and coordinated change in the concentrations of many plasma proteins.
- The concentrations of acute phase proteins do not all increase and decrease in concert.
- Some, for example C-reactive protein (CRP).
- An increased loss of protein can occur in kidney and intestinal diseases.
- In these cases, the proportional loss of low *M*r proteins is greatest.
- A less common cause of a lower concentration of a plasma protein is a reduction in the rate of its synthesis by the liver.
- For example, in cirrhosis of the liver the capacity of the organ to synthesize proteins is reduced.

5

C-reactive protein (CRP)

- A **c-reactive protein** test checks for inflammation in the body. Inflammation can be caused by infection, injury, or chronic disease.
- A high CRP test result is a sign of **acute inflammation**. It may be due to serious infection, injury or chronic disease.
- C-reactive protein (CRP), are part of the innate immune system and its concentration increases rapidly to a thousand-fold the concentration found in the absence of disease.
- It can also decrease just as rapidly.
- C-reactive protein has a direct antibacterial activity.
- A CRP test result of more than 50 mg/dL is generally considered severe elevation. Results over 50 mg/L are associated with acute bacterial infections about 90% of the time.



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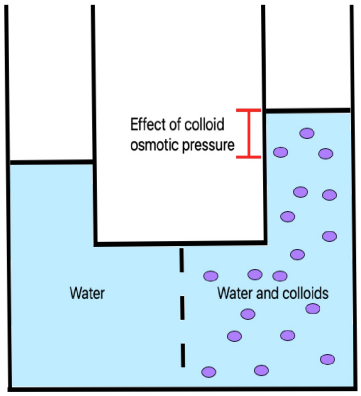
Plasma proteins; Albumin

- Albumin is the major protein product of the liver.
- It has a long biological half-life in plasma (about 20 days).
- ***Hypo-albuminaemia*** is a feature of advanced chronic liver disease.
- Albumin consists of a single polypeptide of 584 residues with a *M*r of 69,000.
- Albumin has two major functions: it is a trans- port protein *par excellence* and it also serves to maintain the **oncotic pressure** of the blood.
- It can also occur in *severe* acute liver damage.



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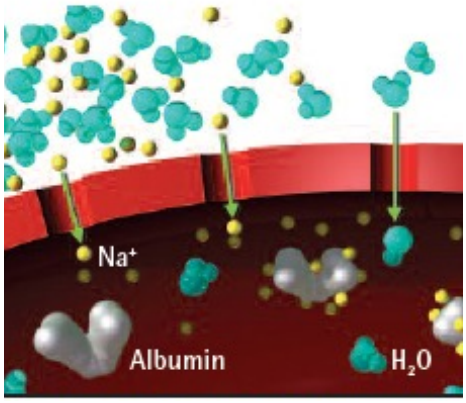
Why albumin concentration is low in blood ?

- A number of pathological conditions result in low concentrations of albumin in the blood.
- These conditions include:
- An acute phase reaction.
- Liver disease.
- Malnutrition
- Malabsorption
- Others



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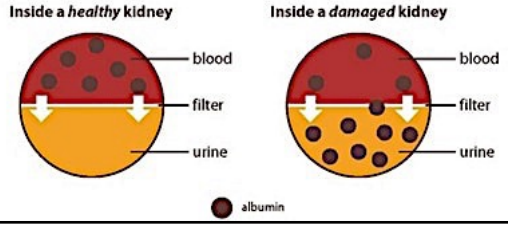
Clinical use of urine albumin measurements

- Albumin is the principal protein present in urine and is the one detected by dipstick tests for urine protein.
- Concentrations lower than about 30 mg/L can be found in the urine of healthy individuals but higher values are a feature of renal disease.
- The limit of sensitivity for urine testing for protein is 250 mg/L.
- However, values lower than 250 mg/L may be associated with disease, a condition termed **microalbuminuria**, which describes urine albumin concentrations 30–250 mg/L.



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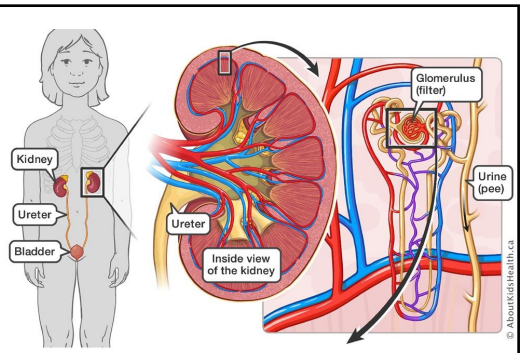
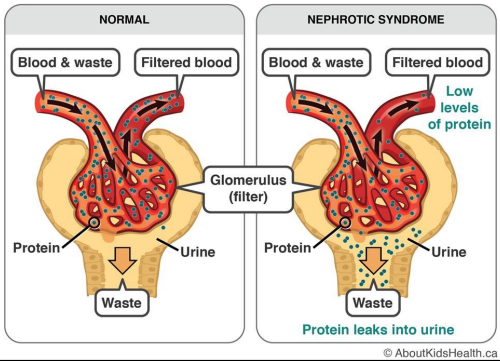
High or low

- **Albuminuria is a sign of kidney disease** and means that you have too much albumin in your urine.
- Albumin is a protein found in the blood. A healthy kidney doesn't let albumin pass from the blood into the urine.
- A damaged kidney lets some albumin pass into the urine.
- Low albumin levels **can be a sign of liver or kidney disease or another medical condition**. High levels may be a sign of dehydration. Albumin is a protein made by your liver.





10

The concentration of albumin in urine

- Measuring the concentration of urine albumin is particularly useful in monitoring diabetes.
- Diabetics are susceptible to a number of complications, one of which is kidney disease.
- Urine output varies to a great extent depending on the amount of fluid a particular individual drinks.
- The concentration of albumin in urine will reflect this, being lower if urine output is high (the urine is dilute) or high when urine output is low (the urine is concentrated).
- To compensate for this the albumin: creatinine ratio is used. Or, collecting urine for 24 hours is a lot less convenient for the patient or

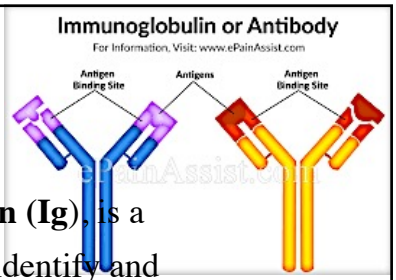
11

What about globulin concentrations?

- The total serum globulin concentrations is sometimes used as a crude measure of the severity of liver disease.
- The immune system is a complex and integrated system of organs, tissues, cells, and cell products, including antibodies and complement proteins.
- In blood, the immune system consists of white blood cells (neutrophils, lymphocytes, and monocytes), antibodies, and another group of plasma proteins called complement proteins.

12

Immunoglobulin

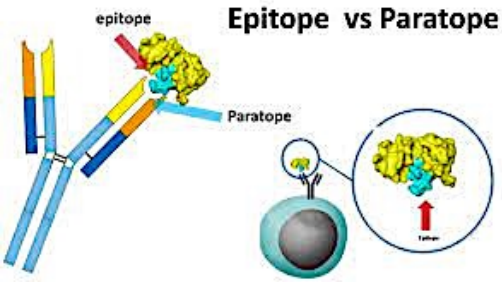


- **An antibody (Ab), also known as an immunoglobulin (Ig)**, is a large, Y-shaped protein used by the immune system to identify and neutralize foreign objects such as pathogenic bacteria and viruses.
- Antibodies **or** immunoglobulins and complement proteins are circulating soluble proteins.
- The immune system recognizes and fights foreign substances and cells, and so protects the body against diseases by killing pathogens such as viruses, bacteria and fungi, and tumor cells.

13

Antibody & antigen

- **Do you remember from immunology !**
- The molecule to which the antibody binds is called an **antigen**.
- The binding site on the antibody is only large enough to bind to a small part of the surface of its cognate antigen.



- It is roughly equivalent to the area occupied by eight amino acid residues.
- The site on the antigen to which the antibody binds is called an **epitope**.

14

Types of Immunoglobulins

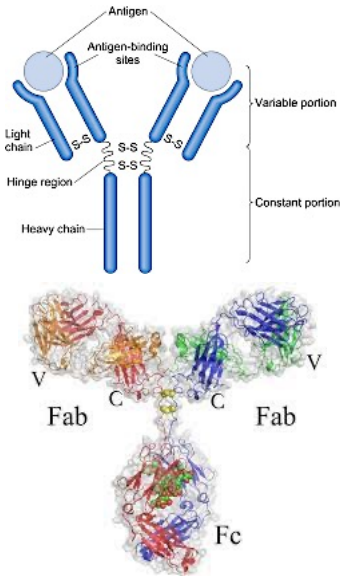
- Immunoglobulins are divided into five classes: IgG, IgA, IgM, IgD, and IgE.
- Of these, only IgG, A, and M are present in plasma at appreciable concentration.

Class	Approximate concentration in serum (mg/L	Adult reference range (g/L)
IgG	10,000	6.0–13.0
IgA	2,000	0.8–3.7
IgM	1,000	0.4–2.2
IgD	50	
IgE	0.1	

15

Structure of immunoglobulin

- All **immunoglobulin** molecules consist of the same basic unit of two heavy and two light chains .
- The heavy chain consists of four domains, three of which are constant and one that is variable.
- The light chain consists of two domains, one is constant and one variable.
- The heavy and light chains associate to form three functional sections: two 'arms' each containing a binding site that is unique for a specific epitope and a 'tail' that is common to all immunoglobulins within a class.



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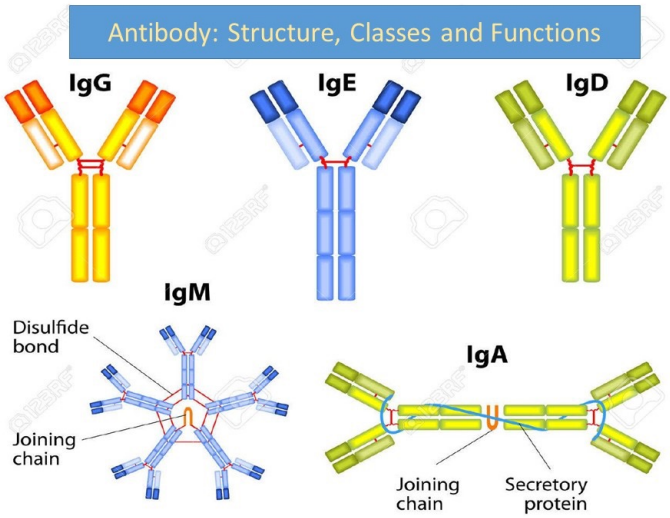
Structure of all types of immunoglobulins



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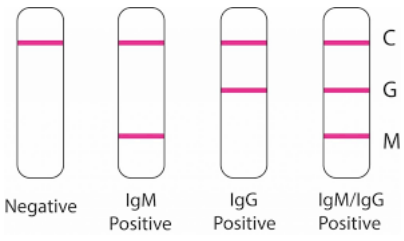
How to find the types of Immunoglobulins in blood

- The body contains many different clones of plasma cells.
- immunoglobulins have the capacity to bind to a vast number of different epitopes on protein and carbohydrate molecules.
- Plasma cells are derived from B lymphocytes which circulate around the body.
- Each B cell has a unique receptor on its cell surface, which is a surface bound form of IgM.
- The receptor has the same binding site as the antibody that the plasma cell derived from the B lymphocyte is destined to produce.



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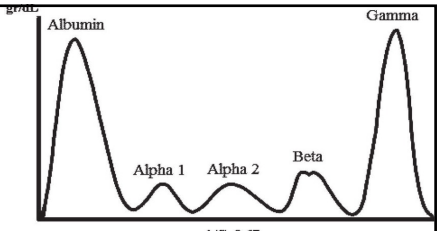
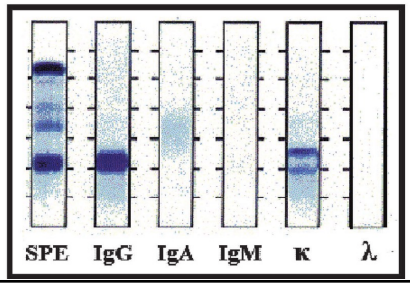
How to detect the levels of globulin?

- **Total protein test:** This blood test measures the total amount of protein in the blood.
- **Serum protein electrophoresis**:
- This blood test measures the level of each type of protein in your blood, including the types of globulins.
- It is a Quantitative method to detect the levels of immunoglobulins.



A/G 0.67
T.P.: 8.2

Fractions	%	Ref.%	gr/dL	Ref. gr/dL
Albumin	36.4	52.9 - 66.9	3.1 L	3.7 - 4.9
Alpha 1	4.4	3.0 - 5.8	0.3	0.2 - 0.4
Alpha 2	11.4	7.5 - 13.4	0.9	0.5 - 0.9
Beta	7.5	8.5 - 13.7	0.5	0.6 - 1.0
Gamma	40.2	8.8 - 19.2	3.6 H	0.6 - 1.4



19

Acute renal failure

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1

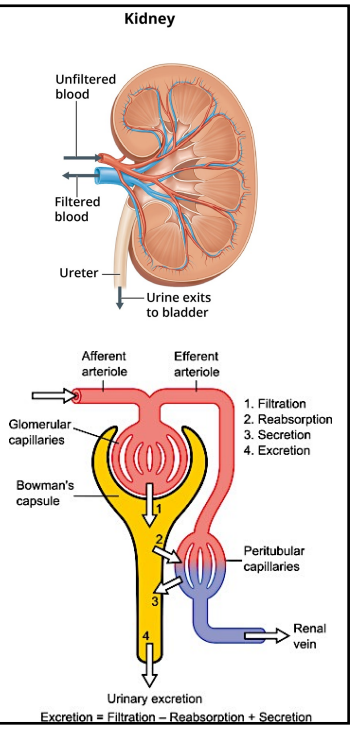
Kidney function tests

- **The renal failure can be detected using specific biochemical parameters such as:-**
- *Creatinine*, *urea*, *uric acid and electrolytes* which are markers of kidney function and the level of electrolytes.
- The parameters are used to diagnose renal (kidney) diseases, but they are also invaluable (important) determining dehydration and cardiac risk.

2

The kidney has three main roles:

- 1. **Blood pressure** and **urine regulation** primarily using the water driving electrolyte sodium (Na), by regulation of water, electrolyte and acid–base balance.
- 2. **Exocrine** such as red cell production through EPO, a hormone made in kidney, and bone metabolism through production of calcitriol (vitamin D metabolite).
- 3. **Excretion** of metabolites such as urea, creatinine and uric acid (a cause of gout).
- The kidneys are also endocrine organs, producing a number of hormones, and are subject to control by others.



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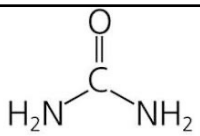
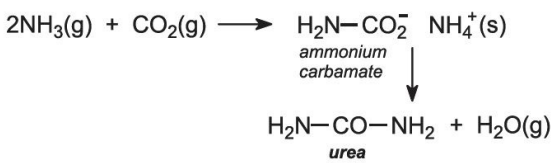
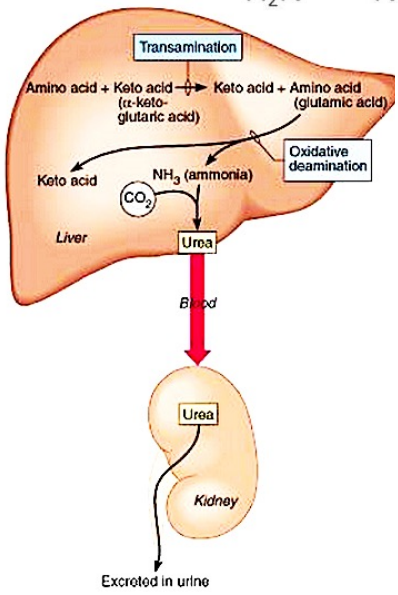
Renal function tests; Serum Urea



- Urea is the major end product of protein metabolism in human body.
- Urea is synthesized in the liver by urea cycle and is excreted by the kidney.
- Urea constitutes the major non-protein nitrogen (NPN) of the blood.
- It represents 45-50% NPN of the blood.
- It is also the major NPN substance excreted in the urine.
- In some countries blood urea is represented as blood urea nitrogen (BUN).





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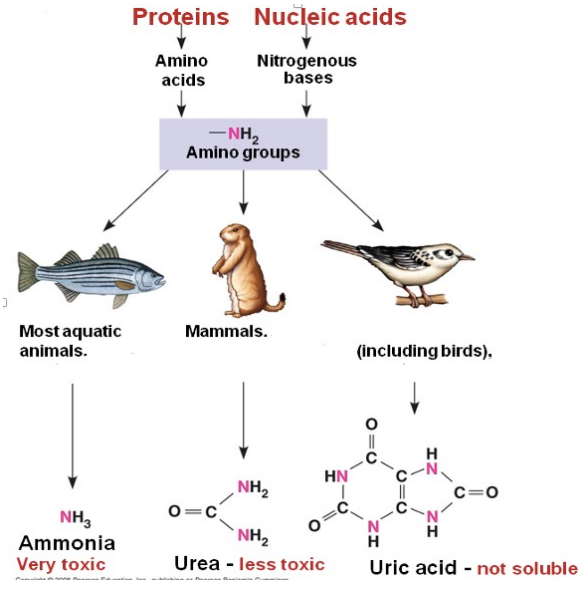
What does serum urea indicate?

- The blood urea, nitrogen test, which is also called a BUN or serum BUN test, **measures how much of the waste product in the blood**.
- If the levels are off the normal range, this could mean that either the kidneys or the liver may not be working properly.
- The normal range of urea nitrogen in blood or serum is 5 to 20 mg/dl, or 1.8 to 7.1 mmol urea per liter.
- The range is wide because of normal variations due to protein intake, endogenous protein catabolism, state of hydration, hepatic urea synthesis, and renal urea excretion.

5

Nitrogenous wasts

- **Key Points**
- Nitrogenous wastes in the body tend to form toxic ammonia, which must be excreted.
- Mammals such as humans excrete urea.
- While birds produce uric acid as waste.
- Conversion of ammonia into uric acid needs more energy than the soppiest.
- Producing uric acid instead of urea is less toxic and reduces water loss and the subsequent need for water.



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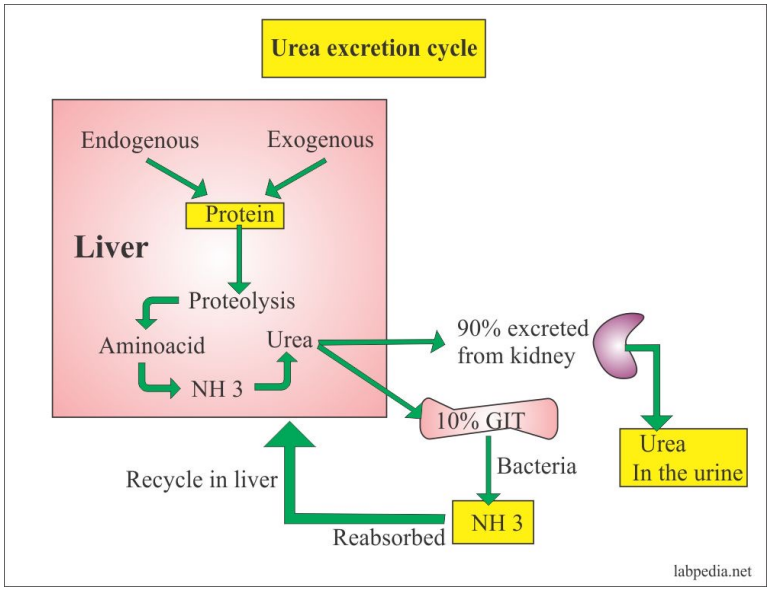
Urea excertion cycle



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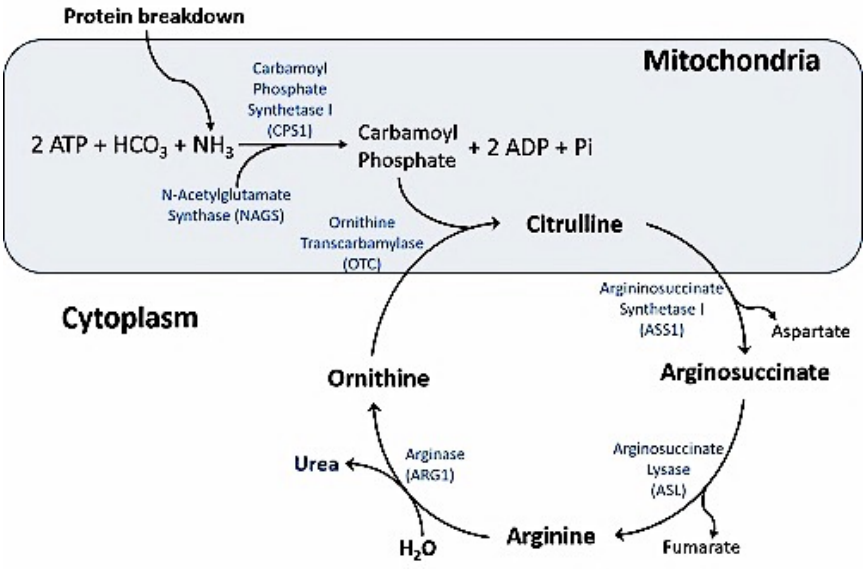
Urea cycle



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Clinical significance of Blood Urea

- Determination of Blood Urea Nitrogen BUN is used in diagnosis of renal insufficiency.
- A BUN of 50 –150 mg/dl implies serious impairment of renal function.
- Markedly increased BUN (150-250 mg/dl) indicates severely impaired glomerular function.
- BUN is also increased in Hemorrhage to GI tract, stress, shock, congestive heart failure, acute myocardial infarction, vomiting-diarrhea etc.
- BUN is found to be lowered in over hydration, severe liver damage, increased utilization of proteins for synthesis, malnutrition, low protein diet, poisoning, hepatitis etc.

9

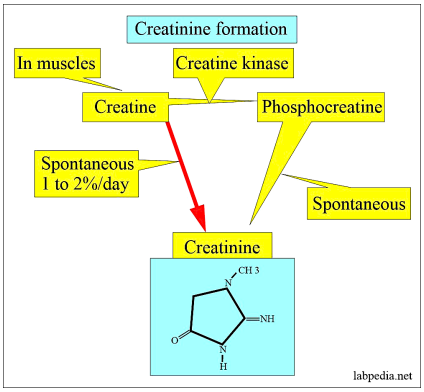
Urea can be estimated by following methods:

- I. Rate of reaction method – UV Kinetic
- II. Berthelot reaction method (end point reaction).
- III. Di-acetylmonoxime method (DAM method)
- When urea standard is used, the value comes out is blood urea and if urea nitrogen standard is used the value is in terms of BUN. The values are converted by:
  BUN = mg % Urea × 0.467
  and mg % Urea = BUN × 2.14

10

Renal function tests; Serum creatinine

- What is Creatine & Creatinine?
- **Creatine** is a molecule that is produced in the body from amino acids when food is converted into energy through a process called metabolism.
- It is present in muscle, brain and blood in free form as well as in the form of creatine phosphate.
- While **Creatinine** is a chemical waste compound that is excreted from the body in urine. It is a waste product creatinine levels is measured to monitor kidney function.
- Creatinine is largely formed in muscle by creatine.



11

When kidneys disunction?

- Creatinine is produced at a "steady rate" and is affected very little by diet or normal physical activities.
- The glomerular filtration rate (GFR) is approximately 140 mL(plasma)/min in a healthy adult.
- If the kidneys are damaged and cannot function normally, the amount of creatinine in the urine decreases while the amount of creatinine in the blood increases.
- Thus, estimation of creatinine directly reflects the kidney function.

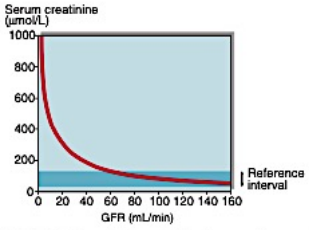
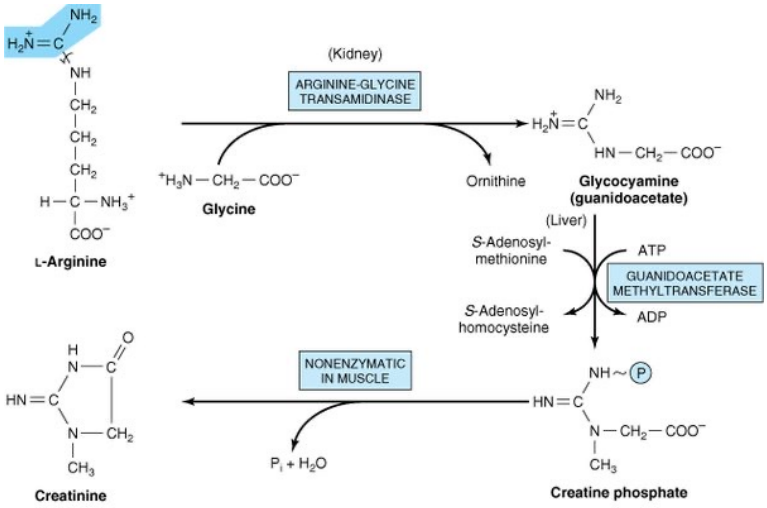


Fig 14.3 **The relationship between glomerular filtration rate and serum creatinine concentration.** Glomerular filtration rate may fall considerably before serum creatinine is significantly increased.

12

The pathway for creatine synthesis, and its conversion to creatinine

- Creatine synthesis requires three amino acids, methionine, glycine, and arginine.
- Also, it requires two enzymes, l-arginine:-glycine amidinotransferase (AGAT), guanidinoacetate methyltransferase (GAMT).



13

Another examination: creatinine clearance

- Creatinine clearance: test measures how well creatinine is removed from blood by the kidneys.
- The creatinine clearance test checks the kidney function by looking at the amount of creatinine in urine and blood over a time and volume , (say, V litres in 24 hours).
- Urine creatinine concentration (U).
- Plasma creatinine concentration (P).
- This is done by as below:
- Normal Value:
  Clearances vary with body weight. It is generally expressed as 1.73m2 of the body surface area.
  Males — 95 - 140 ml/min.
  Females — 85 - 125 ml/min.

$$V = \frac{\text{Total volume of urine in ml}}{\text{Time of collection in minute}} \qquad \text{Creatinine clearance} = \frac{U \times V}{P}$$

14

Determination of renal function

- Serum **creatinine** concentration is an important index of renal function.
- **Proteinuria** may be used as a marker of renal damage and predicts its progression.
- The presence of specific small proteins in urine indicates tubular damage.
- Chemical analysis of **renal stones** is important in the investigation of their etiology.
- Urine test is important as well.

15

Specific kidney disease

- The major causes of kidney disease:

1. Diabetic Nephropathy (is the stop of kidney function).
2. Hypertension and the Kidney.
3. Glomerular Diseases.
4. Chronic renal failure. Renal failure is the stop of kidney function.
5. Others

16

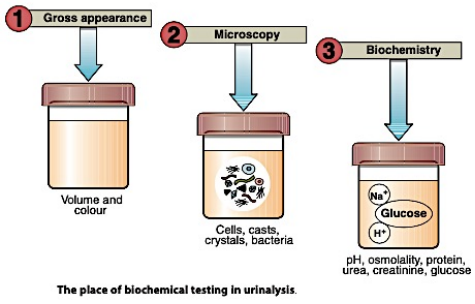
Specific kidney disease

- In patients with both hypertension and proteinuria there is an increased risk of death.
- Plasma sodium, potassium, and creatinine concentrations should be measured to detect **hypokalaemia** (potassium decrease), which could suggest hyperaldosteronism, including Conn's syndrome, and to assess kidney function.

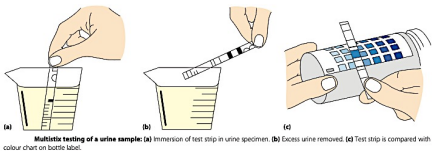
17

Urinalysis

- General urine examination GUE (chemistry tests only)
- Urinalysis should be part of the clinical examination of every patient.
- Chemical analysis of a urine specimen is carried out using commercially available disposable strips.
- The range of components routinely tested for includes glucose, bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrite and leucocytes.
- Chemical examination of urine is one aspect of urinalysis.



The place of biochemical testing in urinalysis.

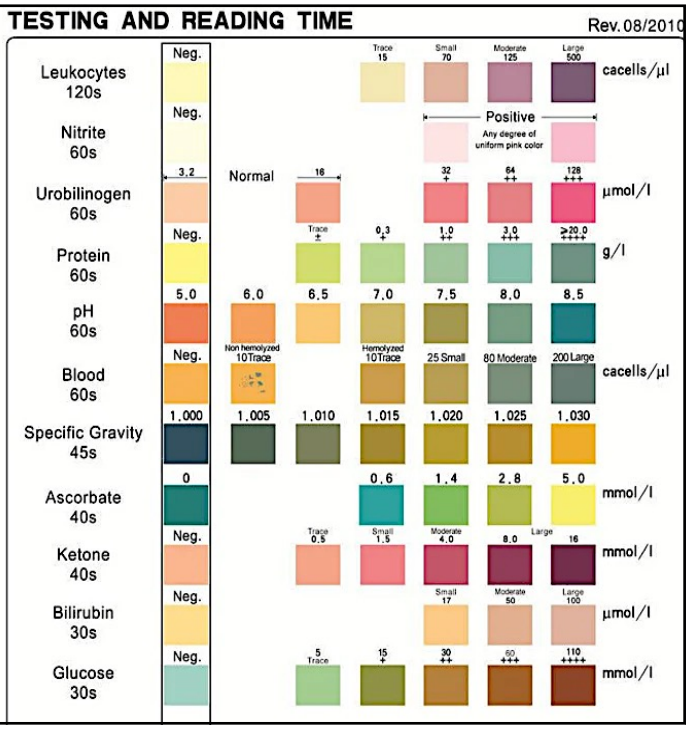

Multistix testing of a urine sample: (a) Immersion of test strip in urine specimen. (b) Excess urine removed. (c) Test strip is compared with colour chart on bottle label.

18

Urinalysis: chemical tests!

- Examination of a patient's urine should not be restricted to biochemical tests. Figure summarizes the different ways urine may be examined.

1. **Bilirubin** some of which spills over into the urine.
2. **Urobilinogen** the finding of urobilinogen in urine is of less diagnostic significance than bilirubin. What is Urobilinogen?
3. **Ketones bodies** are the products of fatty acid breakdown. This can occur in uncontrolled diabetes.
4. **Specific gravity A** higher specific gravity indicates a more concentrated urine.
5. **pH (hydrogen ion concentration)** Urine is usually acidic (urine pH less than 7.4 indicating a high concentration of hydrogen ions).



19

Urinalysis: chemical tests!

6. **Protein: p**roteinuria may signify abnormal excretion of protein by the kidneys (abnormal).
7. **Blood** The presence of blood in the urine is consistent with various possibilities like urinary tract infection UTI and contamination from menstruation.
8. **Nitrite** A positive result points towards a urinary tract infection UTI.
9. **Leucocytes** The presence of leucocytes in the urine suggests acute inflammation and the presence of a urinary tract infection.

20

What other biocmical parametrs ?

- Next …will talk about other biocmical parametrs of kidney fucntion like Uric acid..
- Hormones that affect kidney fucntion.
- Key tests for kidney function.
- Kidney dialysis.

21

Renal Function Test
Uric acid

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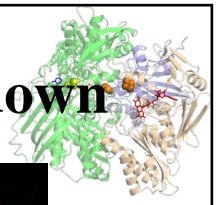
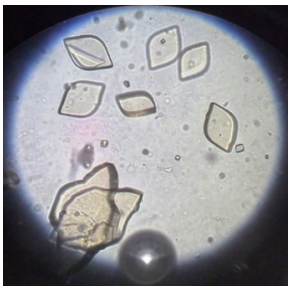
1

Uric acid is a product of DNA breakdown



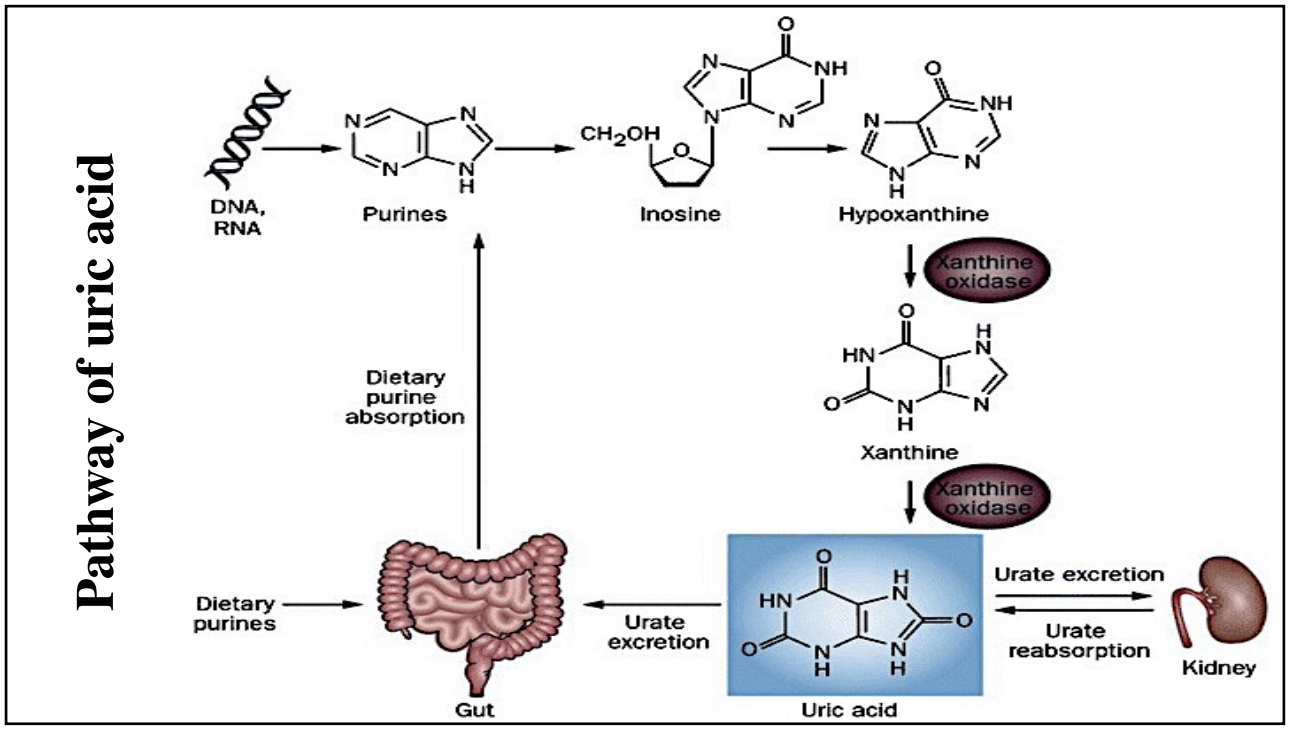
- Uric acid (urate) is a product of DNA breakdown and is normally excreted by the kidney.
- Uric acid concentration rises in renal dysfunction with a low GFR.
- Urate at high concentrations is not soluble.
- The insoluble crystals accumulate joints in hand and feet and can cause gout.

Urate stones



Uric acids under microscopy

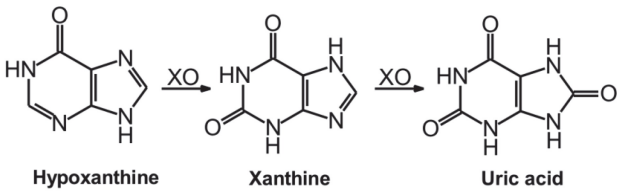
2



3

Uric acid?

- Uric acid is the end-product of purine metabolism.
- The first step in the catabolism of purines (adenine and guanine) is their hydrolytic process called ‘’ deamination’’ to form xanthine and hypoxanthine.
- These products are then oxidized to uric acid.
- Uric acid is filtered in the glomeruli and partially reabsorbed by the tubules and then it is excreted in urine.



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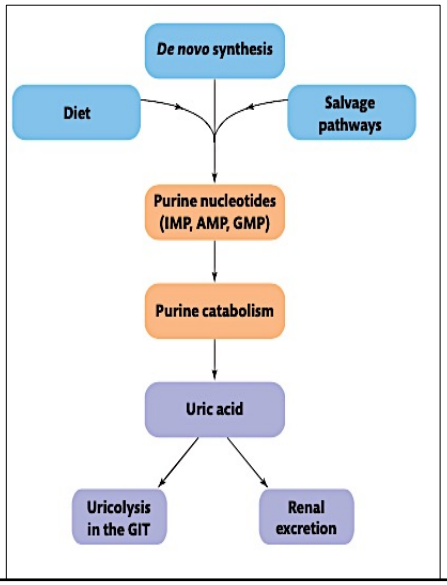
Urate formation and excretion

- Urate is formed in three ways:
- These are:

1. By de novo synthesis
2. By the metabolism of endogenous DNA, RNA and other purine-containing molecules such as ATP.
3. By the breakdown of dietary nucleic acids.

- Urate is excreted in two ways:

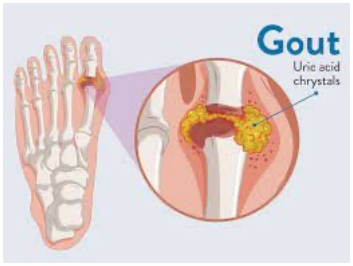
1. *Via the kidney.*
2. *Via the gut by (uricolysis).*

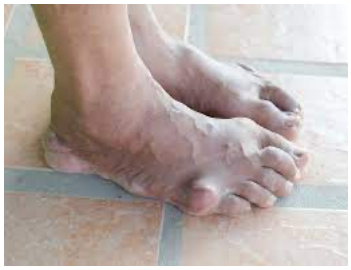


5

Clinical Significance; an increase of uric acid

- Uric acid levels are very changeable and show day-to-day and seasonal variation in some persons.
- It is also increased by emotional stress, total fasting, increased body weight, and renal failure.
- It is found to be increased mainly in ‘’gout’’.
- Also, it is increased leukemia, polycythemia, anemia, psoriasis, hypo and hyperparathyroidism.





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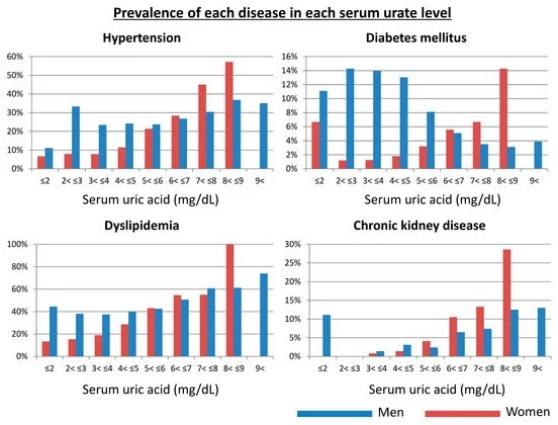
Also,….

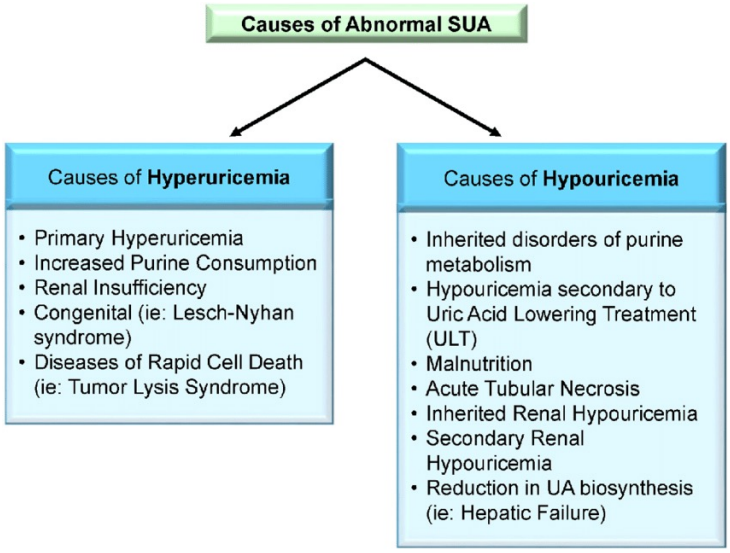
- It is increased with high protein, weight reduction diet, alcohol consumption, arteriosclerosis and hypertension.
- Serum uric acid is increased in 80% patients with elevated serum triglycerides.



7

Clinical Significance; a decrease of uric acid

- Serum uric acid SUA levels are decreased in—Wilson's disease, Fanconi's syndrome, carcinomas, Hodgkin's disease.
- It is also low in healthy adults with isolated defect in tubular transport of uric acid.



8

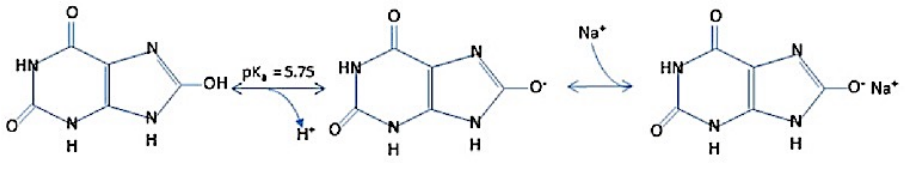
Purines metabolism

- Purine nucleotides are catabolized (degraded) by reactions that initially form their respective nucleosides: inosine, adenosine, guanosine, and xanthosine.
- The commonly found nucleotides are converted by enzymatic reactions into xanthine.
- In turn, xanthine is oxidized to uric acid.
- Consequently, uric acid is the main end product of metabolism and the degradation of purines, leads to the formation of uric acid.

9

How do kidneys get rid of uric acid?

- Uric acid is sparingly soluble but, despite this, the kidneys play the major role in removing it from the blood.
- This is possible because it can ionize in the presence of sodium, giving the salt, monosodium urate as shown in the following slide.
- Monosodium urate is generally referred to as uric acid in clinical environments and it will adhere to this convention.



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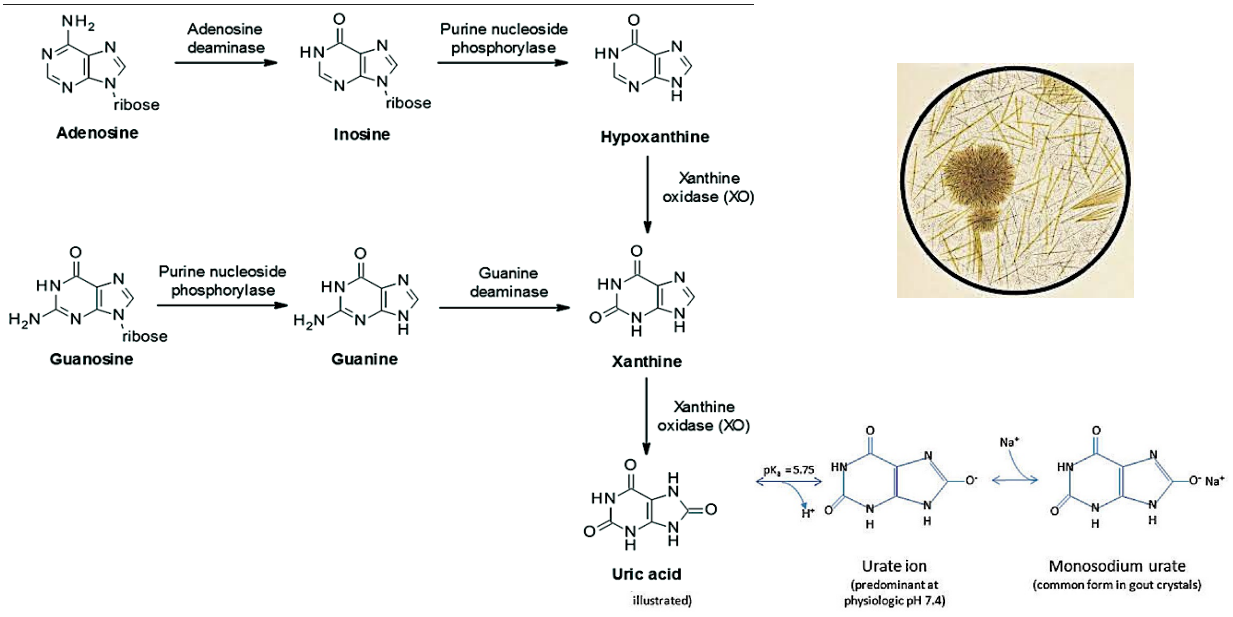
Monosodium urate pathway



11

The fate of uric acid

- Approximately 98% of the uric acid filtered by the glomeruli is reabsorbed by the proximal tubules.
- Despite this, about two-thirds to three-quarters of the uric acid excreted is eliminated by the kidneys.
- The remaining uric acid is secreted into the gastrointestinal tract (GIT or called also GUT) where it is metabolized by gut bacteria in a process called **uricolysis** to form carbon dioxide and ammonia:
    - Uric acid + 4 H2O + → 4 NH4+ + 3 CO2 + glyoxylic acid

12

Hypouricemia and hyperuricemia

- The concentrations of uric acid in blood are normally determined in samples of serum.
- Concentrations are generally higher in males than females, with a usual reference range for males of 0.1–0.42 and for females 0.1–0.36 mmol/L.
- Values below or above these limits are called hypouricaemia and hyperuricaemia respectively.

Normal value:

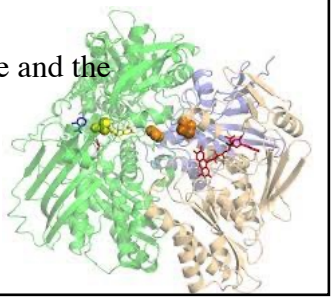
1 – 3 yr	1.8 – 5 mg/dl
4 – 13 yr	2.2 – 4.7 mg/dl
14 – 19 yr	2 – 5 mg/dl

	Males	Females
10 – 11 yr	2.3 – 5.4 mg/dl	3 – 4.7 mg/dl
12 – 13 yr	2.7 – 6.7 mg/dl	3 – 5.8 mg/dl
14 – 15 yr	2.4 – 7.8 mg/dl	3 – 5.8 mg/dl
16 – 19 yr	4 – 8.6 mg/dl	3 – 5.9 mg/dl

13

Hypouricemia (-uricaemia)

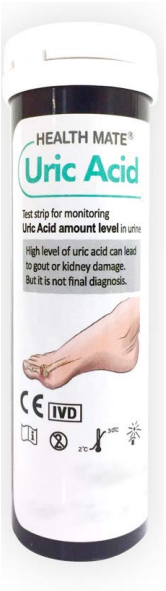
- Hypouricemia is a measured serum concentration of uric acid below its reference ranges.
- It is rare.
- The primary metabolic cause is a deficiency of xanthine oxidase activity in the liver.
- This results in increased excretions of xanthine and hypoxanthine and the formation of xanthine stones.
- This condition is called xanthinuria.



14

Hyperuricemia

- Hyperuricemia is defined as an increase in the concentration of uric acid above the reference ranges.
- The concentration of uric acid in plasma reflects an equilibrium between the amount ingested and produced, and the quantity excreted.
- The causes of hyperuricemia are primary disorders due to inherited metabolic diseases and secondary ones caused by a co-existing clinical condition.
- Both, of course, lead to an accumulation of uric acid in the body.



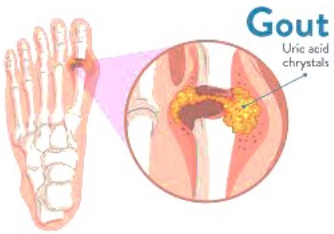
15

Hyperuricemia

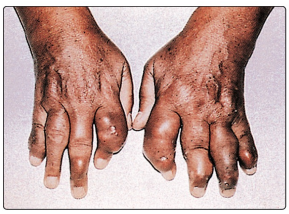
- Nucleic acids contain bases of two different types, pyrimidines and purines.
- The catabolism of the purines, adenine and guanine, produces uric acid.
- At physiological hydrogen ion concentration, uric acid is mostly ionized and present in plasma as sodium urate.

16

What is Gout?



- Gout is a clinical syndrome that is characterized by hyperuricemia an recurrent acute arthritis.
- Whereas all patients who develop gout will have had hyperuricemia a point.
- Only a minority of patients with hyperuricemia develop gout.
- The reason for this is not known.
- Acute gout is triggered by the tissue deposition of sodium urate crystals t cause an intense inflammatory response.
- Gout is exacerbated by alcohol. The reason for this is twofold. Ethanol increases the turnover of ATP and urate production.

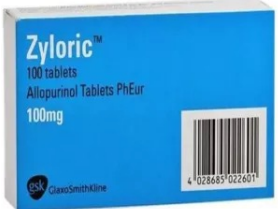


Tophaceous deposits of sodium urate in tissues.

17

GOUT Treatment by Allopurinal

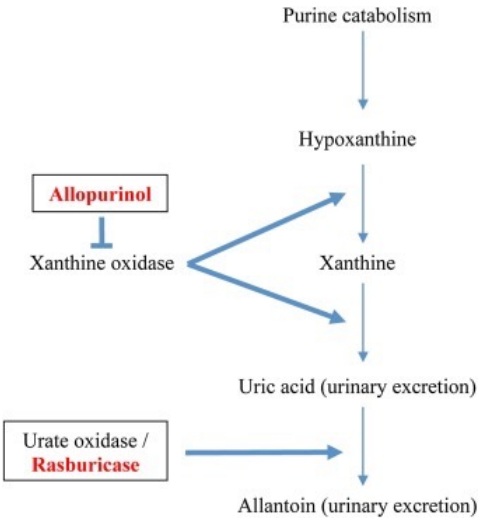
- The symptoms of acute gout respond to anti-inflammatory drugs such as indomethacin, but it should be noted that these drugs have no direct effect on the serum urate level.
- To assist in differentiating the types of gouts, Allopurinal (100-300 mg) xyloric@ treatment can reduce urate gout,



18

Mechanisim of Allopurinol on GOUT

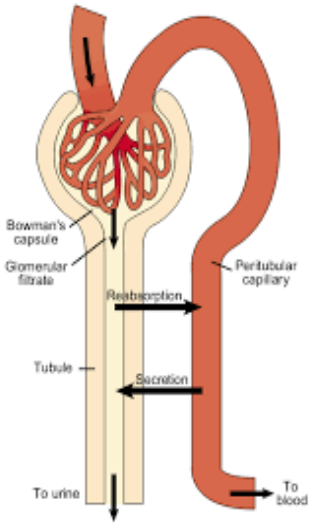
- Allopurinol, a specific inhibitor of the enzyme xanthine oxidase that catalyzes the oxidation of hypoxanhine to xanthine and uric acid, can also be effective in reducing urate concentrations.
- A number of other crystalline arthropathies may present as gout but are not associated with hyperuricaemia (so-called *pseudogout*).



19

Renal disease and hyperuricemia

- Renal disease is a common complication of hyperuricemia.
- Several types of renal disease have been identified.
- The most common is urate nephropathy, which is caused by the deposition of urate crystals in renal tissue or the urinary tract to form urate stones.
- Acute renal failure can be caused by the rapid precipitation of uric acid crystals that commonly occurs during treatment of patients with leukemias and lymphomas.



20

Calcium regulation and vitamin D3

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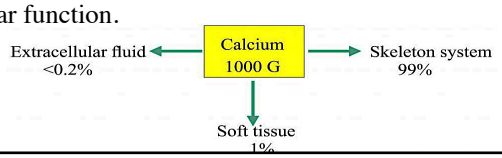
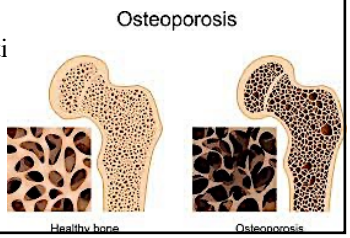
1

Calcium and bones

- Calcium is the most important and essential mineral.
- Calcium and phosphorus are the main bases of bones, which make up the intercellular matrix of bone structure.
- Calcium and phosphorus are continually stored in bone and resorbed into blood as nutrients are available.
- Calcium involves 99 % the body's bones.
- The remaining 1 % free in circulation of the plasma.
- Of this remaining 1 %, half is bound to albumin and half is actively precipitati cellular function.





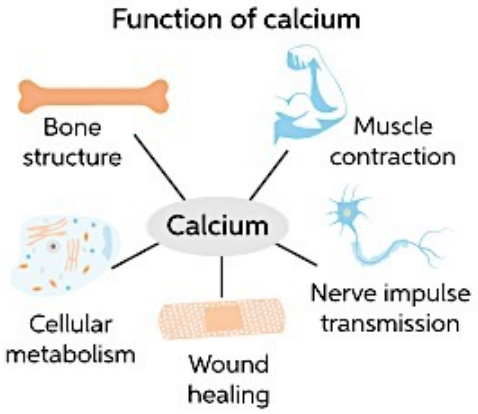
2

Function of Calcium

- Calcium performs mainly the following functions:

1. Builds and repair bones and teeth.
2. Assists cellular metabolism.
3. Assists nerve transmission.
4. Helps muscle function.
5. Helps wound healing

Calcium could also reduces cancers.



3

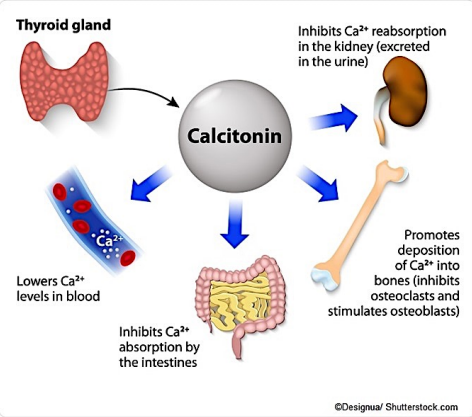
Calcium: Sources, Absorption, Deficiency and toxicity

- **Sources**: the main sources of calcium milk and milk products.
- **Absorption**: Calcium is taken in diet as calcium phosphate, Ca-carbonate Ca-tartarate and Ca-oxlate. Its absorbed mainly in intestine.
- **Deficiency**: May result in arm and leg muscles spasms, softening of bones, back and leg cramps, tooth decay and depression.
- **Toxicity**: Occurs in hypervitaminosis D and hyperthyroidism or idiopathic hypercalcemia (vomiting and pain)…

4

Calcium and phosphate and vit D3

- Analysis of vitamin D concentration provides a measure of the ability of the body to absorb dietary calcium through the small intestine.
- Serum calcium and phosphate concentrations provide a measure of the resorption of these chemicals from bone.



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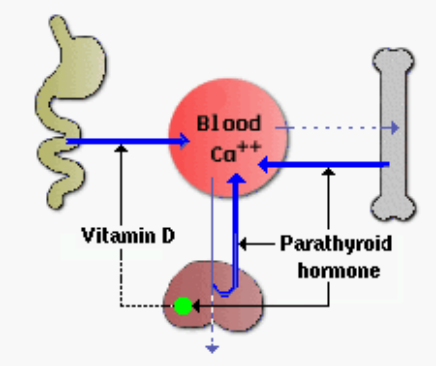
Bones

- The bone therefore has three main roles:

1. storage the calcium and other chemicals.
2. In the bone morrow assisted the production of red and white blood cells.
3. Physical support of the body.



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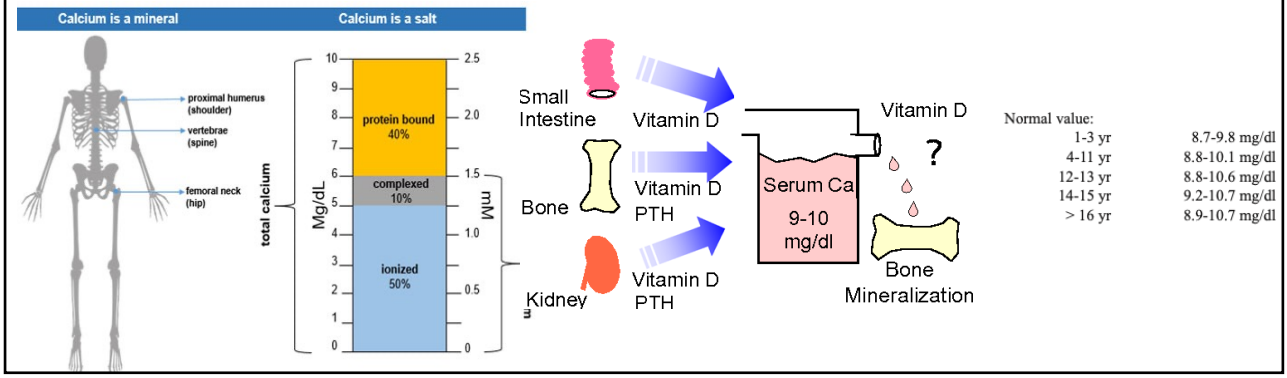
Total serum calcium

- A healthy person has a total serum calcium of around 2.4 mmol/ L.
- About half is bound to protein, mostly to albumin.
- Unbound calcium is the biologically active fraction of the total calcium.



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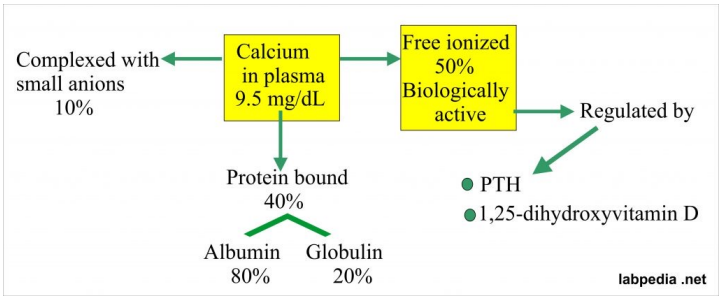
Free calcium and bound calcium

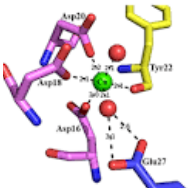
- Calcium is required for nerve function, membrane permeability, muscle contraction and glandular secretion.
- This is the unbound calcium concentration that is recognized by the parathyroid glands, and PTH acts to keep this concentration constant.

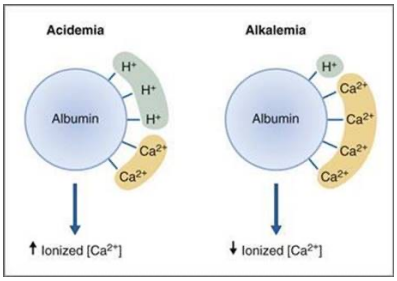


8

Calcium and albumin

- Laboratories routinely measure total calcium concentration (that is both the bound and unbound fractions) in a serum sample.
- However, serum albumin could affect calcium concentration and changes total calcium concentration.
- If albumin concentration falls, total serum calcium is low because the bound fraction is decreased.





9

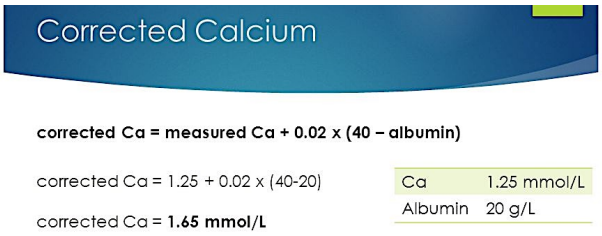
Low albumin !

- Remember that the homeostatic mechanisms for regulating plasma calcium respond to the unbound fraction, not to the total calcium.
- Patients with a low albumin have total serum calcium lower than the reference values,
- Yet, have normal unbound calcium.
- These patients should not be thought of as hypocalcaemic.

10

Adjusted calcium

- Clinical biochemists use the convention of the 'adjusted calcium'.
- Most laboratories measure both total calcium and albumin, and, if the albumin is abnormal, calculate what the total calcium would have been if the albumin had been normal.
- One such calculation is:
- Adjusted calcium (mmol/L) = Total measured calcium + 0.02(47– albumin)



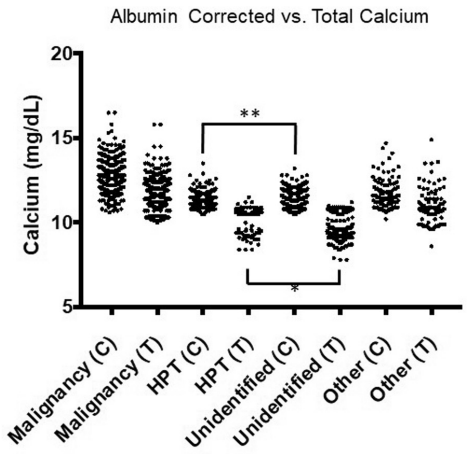
11

Do correction after mesurements!

Corrected Calcium Equation

- **Corrected calcium** mmol/L=
serum calcium mmol/L+ 0.02 (40 - serum albumin g/L)

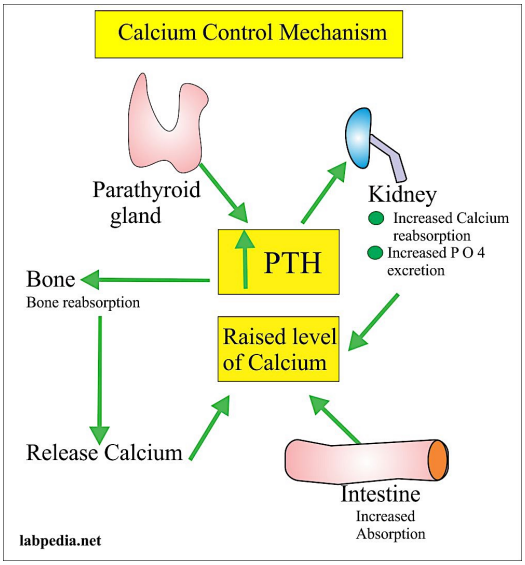
- **Corrected calcium** mg/dl =
serum calcium mg/dl + 0.8 (4 - serum albumin g/dL)



12

Clinical Significance of serum calcium

- Determination of serum calcium level is useful in diagnosis of parathyroid dysfunction, hypercalcemia of malignancy.
- Most cases of hypercalcemia are due to hyperparathyroidism and some other diseases.
- Calcium levels are found to be low in hypoparathyroidism, malabsorption of calcium and vitamin D, chronic renal disease and some other chronic diseases.

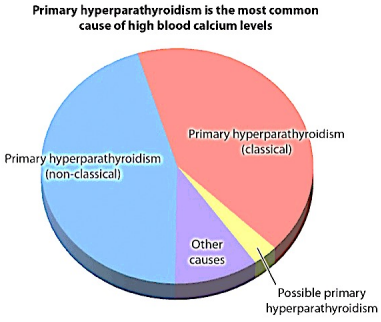


13

Hypocalcaemia

- The causes of hypocalcaemia mainly include:

1. *Vitamin D deficiency*. This may be due to malabsorption, or not enough diet with little exposure to sunlight. It may lead to the bone disorders, osteomalacia in adults and rickets in children.
2. *Hypoparathyroidism*.
3. *Magnesium deficiency*.
4. *Renal disease*.
5. Others.



14

Hypocalcaemia

- The clinical features of hypocalcaemia include associated with albumin:

1. Neurological features such as tingling ‘’ irritated nerve’’.
2. Mental changes.
3. Cardiovascular signs such as an abnormal ECG.

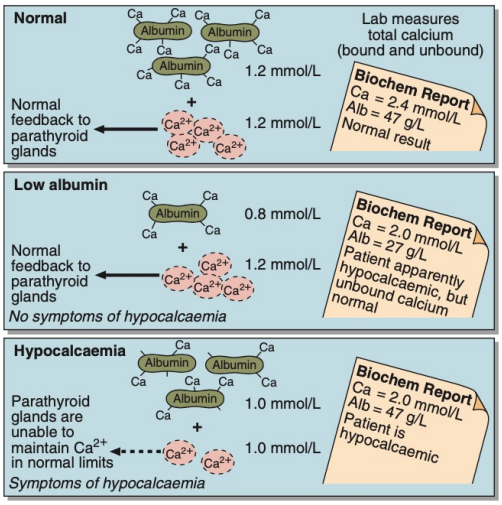


Fig 35.3 **The binding of calcium to albumin.**

15

Dignosis of hypercalcaemia

- The commonest causes of hypercalcaemia are primary hyper- parathyroidism and hypercalcaemia of malignancy.
- Rarer causes of hypercalcaemia include *Inappropriate dosage of vitamin D or metabolites and other disease*.

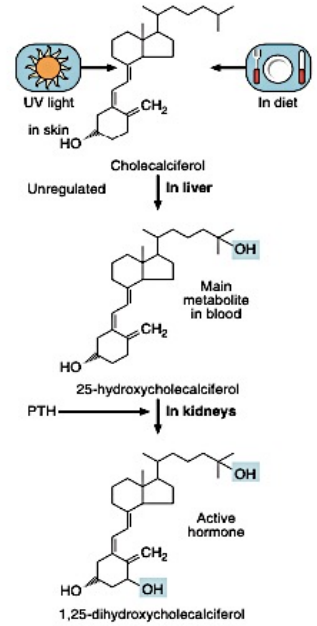


Fig 38.3 **The main steps in the metabolism of vitamin D.**

16

Treatment of hypercalcaemia

- Primary hyperparathyroidism (usually not cancer)
- Treatment is urgent if the adjusted serum calcium is greater than 3.5 mmol/L; the priority is to reduce it to a safe level.
- Intravenous saline is administered first to restore the glomerular filtration rate and promote a diuresis.
- Drugs such as steroids, mithramycin, calcitonin and intravenous phosphate are essential.

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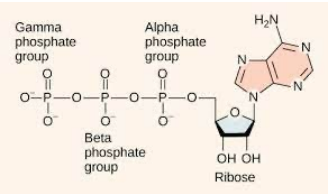
Calcium level in urine

- Blood calcium should be monitored mainly in renal disease.
- Determination of urine calcium level is useful in diagnosis of hypercalciuria causing renal calculi.
- High calcium levels in urine are seen in – hyperparathyroidism, excess milk intake, Paget's disease, etc.
- Low calcium level in urine is due to renal failure, hypoparathyroidism, rickets, osteomalacia and cancers.

18

Phosphate and calcium association

- Phosphate is correlated to Calcium and most of the body's phosphate is in bone.
- Much of the phosphate inside cells is covalently attached to lipids and proteins.
- Enzymes are phosphorylated and dephosphorylated.
- Phosphate changes are associated with PTH.
- Hyperphosphataemia may replace with calcium phosphate leads to high serum phosphate concentration.
- Hypophosphataemia is rare and causes muscle weakness (affect lings).



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Magnesium and calcium association

- Magnesium ions are the second most important intracellular cations, after potassium.
- More than 300 enzyme systems are magnesium activated.
- Magnesium is the bases of intracellular reactions e.g glycolysis.
- Magnesium influences the secretion and the action of PTH.
- Severe hypomagnesaemia may lead to hypoparathyroidism and cause hypocalcaemia.

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Magnesium and phosphate association

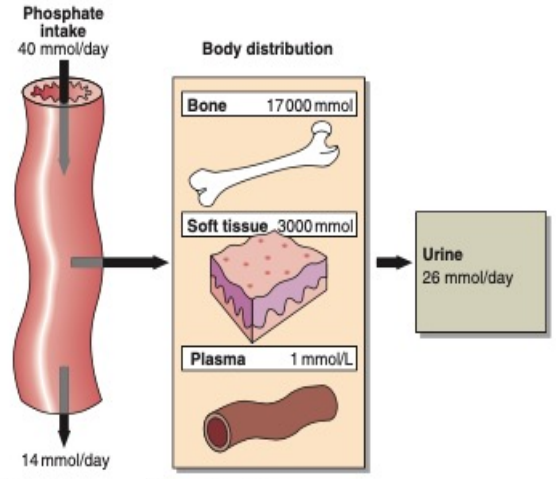


Fig 37.1 **Normal phosphate balance.**

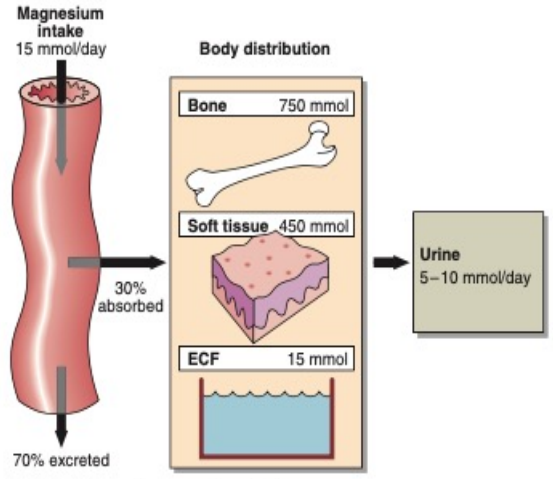
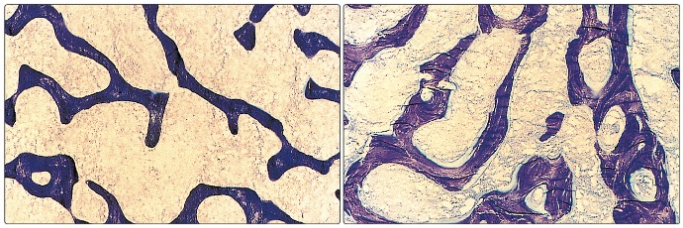


Fig 37.2 **Normal magnesium balance.**

21

Bone disease and biochemistry

- Osteomalacia and rickets Paget's disease.
- **Paget's disease which is** characterized by increased osteoclastic activity.
- Hydroxyproline, from the breakdown of collagen, can be used to monitor bone resorption.



ig 38.2 **Bone biopsy showing normal (left) and osteomalacic (right) bone.**

Table 38.1 **Biochemical profiles in bone diseases**

Disease	Profile
Bone metastases	Calcium may be high, low or normal Phosphate may be high, low or normal PTH is usually low Alkaline phosphatase may be elevated or normal
Osteomalacia/rickets	Calcium will tend to be low, or may be clearly decreased PTH will be elevated 25-hydroxycholecalciferol will be decreased if the disease is due to vitamin D deficiency
Paget's disease	Calcium is normal Alkaline phosphatase is grossly elevated
Osteoporosis	Biochemistry is unremarkable
Renal osteodystrophy	Calcium is decreased PTH is very high
Osteitis fibrosa cystica (primary hyperparathyroidism)	Calcium is elevated Phosphate is low or normal PTH is increased, or clearly detectable and thus 'inappropriate' to the hypercalcaemia

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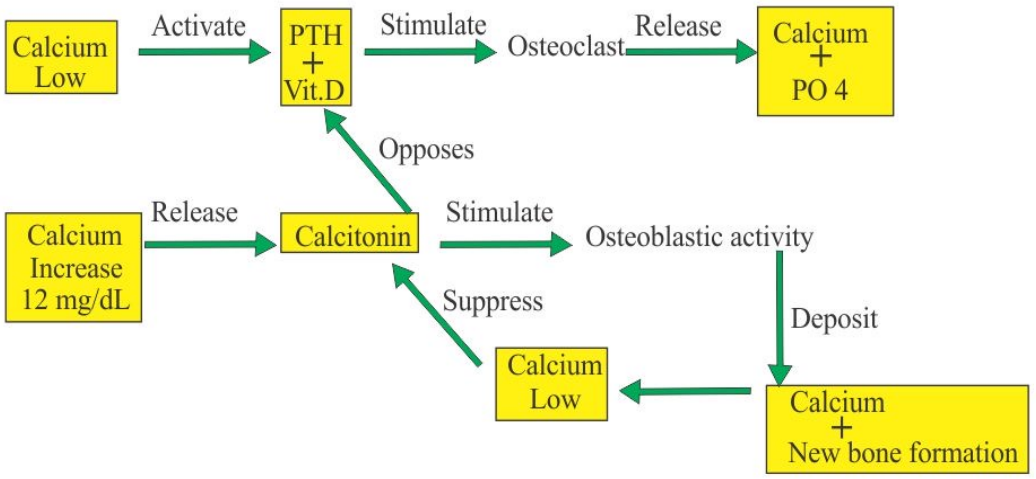
Calcium metabolism



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Clinical significance of phosphorus

- High urinary phosphorus (i.e. increased renal losses) occurs in primary hyper-para-thyroidism, vitamin D deficiency, renal tubular acidosis, diuretic use.
- Renal loss of phosphate may itself lead to rickets or osteomalacia.
- Low in hypoparathyroidism, pseudo-hypo-parathyroidism, vitamin D intoxication.

24

Acids-base, Minerals, and Trace elements disorders

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Department Of Biophysics

1

Terminology

- Acidosis and alkalosis are clinical terms that define the primary acid–base deficiency.
- They can be used even when the [H^+] is within the normal range.
- The definitions are:
- *Metabolic acidosis*. The primary disorder is a decrease in bicarbonate concentration.
- *Metabolic alkalosis*. The primary disorder is an increased bicarbonate.
- *Respiratory acidosis*. The primary disorder is an increased PCO_2.
- *Respiratory alkalosis*. The primary disorder is a decreased PCO2.
- Acidaemia' and 'alkalaemia' refer simply to whether the [H^+] in blood is higher or lower than normal, and the terms are not frequently used.

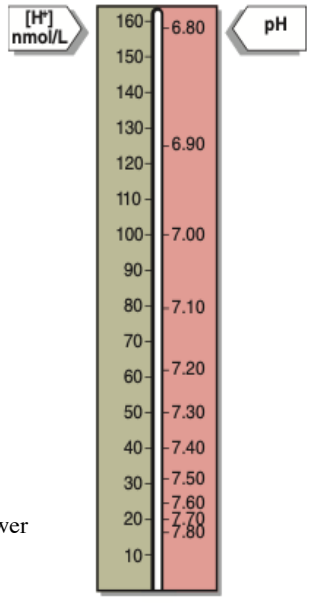
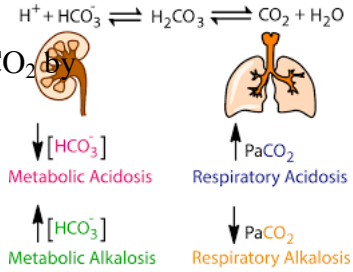


Fig 20.1 **The negative logarithmic relationship between [H^+] and pH.**

2

Metabolic acid–base disorders

- Metabolic acid–base disorders are caused by an increase in H^+ production or a loss of H^+ which results in the loss or gain of $HCO3^-$.
- Direct loss or gain of $HCO3^-$ will also cause metabolic acid-1base disorders.
- Primary metabolic acid– base disorders are recognized by a check of bicarbonate concentration.
- Respiratory exchange helps metabolic acid–base in blood PCO_2 by hyperventilation or hypoventilation.



3

Acid–base disorders

- Metabolic' acid–base disorders are those that directly cause a change in the bicarbonate concentration.
- Respiratory: acid–base disorders affect directly the PCO_2.
- Impaired respiratory function causes a increase of CO_2 in blood.
- Hyperventilation can cause a decreased PCO_2.

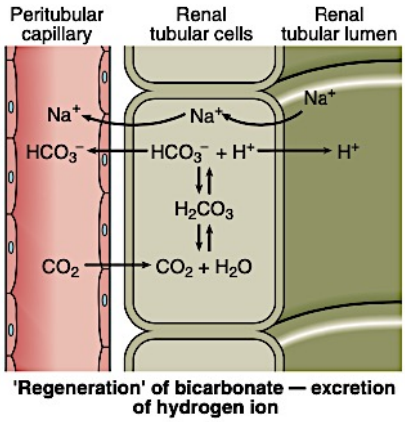
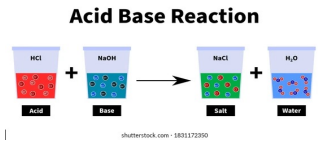


Fig 20.2 **The recovery and regeneration of bicarbonate by excretion of H^+ in the renal tubular cell.** Note that H^+ is actively secreted into the urine while CO_2 diffuses along its concentration gradient.

4

Acid-base concepts

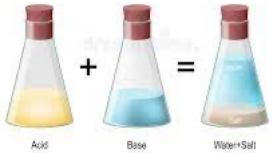


- The measurement of acid–base status is carried out by measuring [H^+], [$HCO3^-$] and PCO2, the components of the bicarbonate buffer system in plasma.
- Adding H^+, removing bicarbonate or increasing the PCO2 will all have the same effect, an increase in [H^+].
- Removing H^+, adding bicarbonate or lowering PCO2 will all cause the [H^+] to fall.
- Primary problems with H^+ production or excretion are reflected in the [$HCO3^-$] and these are called ‘metabolic’ acid–base disorders.

5

Acid-base concepts

- Primary problems with CO2 excretion are reflected in PCO2; these are called ‘respiratory’ acid–base disorders.
- The body has physiological mechanisms that try to restore [H+] to normal.
- These processes are called ‘compensation’.
- The observed [H^+] in any acid–base disorder reflects the balance between the primary disturbance and the amount of con



6

Acid-base concepts

- Blood cancer results and acid base disorders.
- Acidosis and alkalosis you to metabolic acidosis and alkalosis change by respiratory compensation.

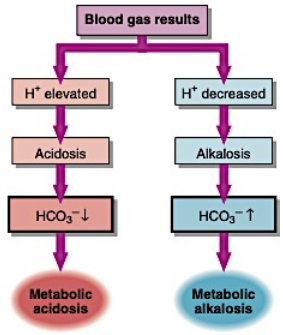


Fig 21.1 **Recognizing primary metabolic acid–base disorders by inspecting the HCO_3^- concentration.**

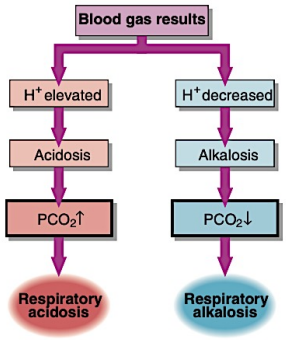


Fig 22.1 **Primary respiratory disorders are recognized by inspecting the PCO_2.**

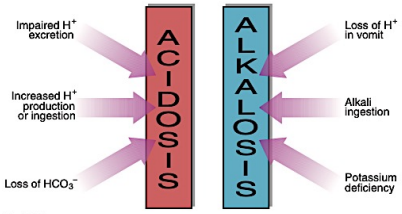


Fig 21.3 **Reasons for metabolic acidosis and alkalosis.**

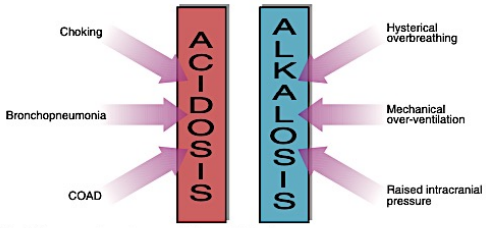


Fig 22.4 **Causes of respiratory acidosis and alkalosis.**

7

Equilibrium of alkalosis and acidosis

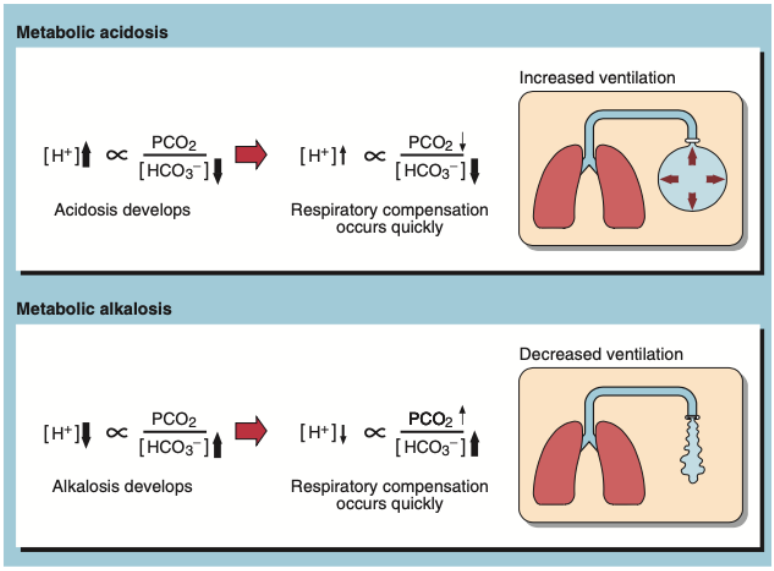
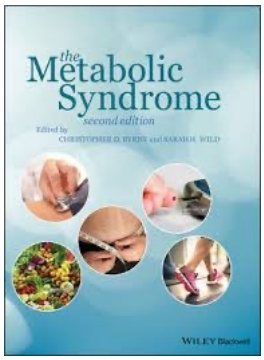


Fig 21.2 **Compensation in primary metabolic disorders.**

8

Metabolic acid–base disorders

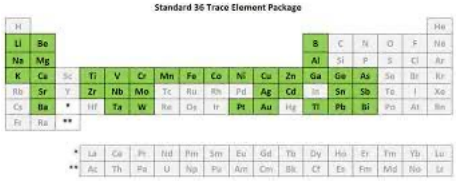
- ***In metabolic acidosis***, the blood [H^+] may be high or normal, but the [$HCO3^-$] is always low. In compensated conditions, PCO2 is lowered.
- The commonest causes of metabolic acidosis are renal disease, diabetic ketoacidosis and lactic acidosis.
- ***In metabolic alkalosis,*** the [H^+] is depressed and the [$HCO3^-$] is always raised. Respiratory compensation results in an elevated PCO2.
- The commonest causes of a metabolic alkalosis are diuretic therapy and prolonged vomiting.



9

Minerals and trace elements

- Minerals are inorganic substances mined from the earth.
- Of the 92 naturally occurring elements, the 14 minerals have been shown by research to be essential to human health are: calcium, chromium, copper, fluorine, iodine, iron, magnesium, manganese, molybdenum, phosphorus, potassium, selenium, sodium and zinc.



10

Trace elements

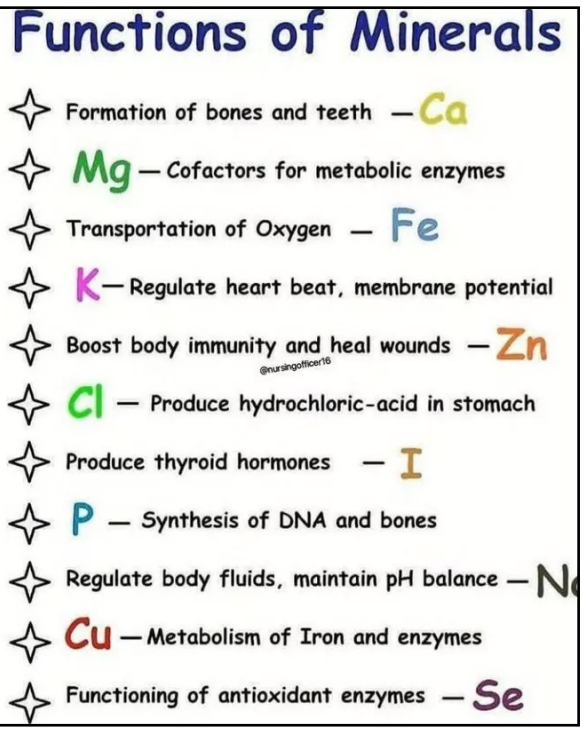
- Essential macrominerals are those needed in significant quantities (such as calcium) – usually measured in milligram.
- Essential trace minerals are needed in minute quantities (such as selenium) – usually measured in micrograms (one microgram [mcg] equals 1/1,000th of a milligram [mg]). See the table in next page

11

Functions of minerals

- Minerals are required for a variety of physiological functions. Their functions are—

1. Maintenance of osmotic pressure of cell
2. Transport of oxygen
3. Growth and maintenance of tissues and bones
4. Working of nervous system
5. Muscle contraction
6. Maintenance of electrolytic balance
7. Acid-base balance



12

The Important Minerals Required in Human Body

The major elements that compose the human body and their relative amounts are as follows:

Mineral content of human Element body	*Percent*	*Approximate amount (in gm) in 70 Kg adult*
Ca^{++}	1.50	1050
P	1.00	700
K^{+}	0.35	245
Na^{++}	0.15	105
Cl^{-}	0.15	105
Mg^{++}	0.05	035
Fe^{++}	0.004	003
Zn^{++}	0.0033	02.3

13

Iron (Fe)

- Iron is an essential constituent of hemoglobin and certain enzymes such as cytochrome oxidase, catalase and peroxidase.
- It transport oxygen to tissues (through Hb hemoglobulin).
- The normal concentration of protein bound iron in plasma is 50 - 180µ gm/ 100ml.
- Daily requirement: Infants – 6–15 mg, Children- 10–18 mg, Adult(male) 10 mg, female- 18 mg.
- Diffecency of iron couse anemia.
- Iron is known in blood as as ferritin.

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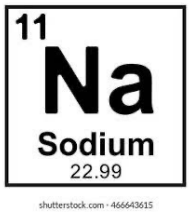
Sodium Na and Potassium K



- **Sodium** is a principal cation in extracellular fluid.
- It regulates plasma volume, acid-base balance etc.
- Metabolism: Metabolism is regulated by aldosterone (progesterone, ACH)
- Deficiency is related to diarrhea, vomiting etc.
- It is characterized by headache, nausea, cramps, fall in BP, oliguria, increased pulse rate.
- **Potassium**: works with sodium to regulate the body's waste balance and normalize heart rhythms.
- Aids in clear thinking by sending oxygen to the brain.
- Plasma level: 3 – 5 mEq/lit. Daily requirement: 4 gm.

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Trace elements Copper and Iodine

- **Copper**: It is necessary for the absorption and utilization of iron; helps oxidize Vitamin C and works with Vitamin C to form Elastin,
- Deficiency: May result in general weakness, impaired respiration, skin sores.
- **Iodine**: Aids in the development and functioning of the thyroid gland.
- Deficiency: May result in an enlarged thyroid gland, slow mental reaction, dry skin and hair, weight gain, loss of physical and mental vigor.
- Toxicity causes thyrotoxicosis and goiter.

16

Manganese and Zinc

- **Manganese**: An antioxidant nutrient; important in the blood breakdown of amino acids and the production of energy.
- Deficiency: May result in dizziness, ataxia, loss of hearing, digestive problems, blindness and deafness in infants.
- **Zinc**: Is an antioxidant nutrient; necessary for protein synthesis; wound healing.
- Deficiency: May result in delayed sexual maturity, and other diseases.

17

Molybdenum, Selenium and Chromium

- **Molybdenum**: is involved in the operation of several key enzymes in the body.
- Deficiencies of this essential mineral are unusual, although rare deficiencies occur in people who suffer from malabsorption conditions.
- Elevated levels of molybdenum can cause a loss of copper.
- **Selenium**: A major antioxidant nutrient, decreasing the risk of cancer and disease of the heart and blood vessels by generation free radicals
- Deficiency: May result in premature aging, heart disease, etc.
- **Chromium**: Works with insulin in the metabolism of sugar and stabilizes blood sugar levels; cleans the arteries by reducing Cholesterol and Triglyceride levels;
- Deficiency: May result in glucose intolerance in diabetics; arteriosclerosis, heart disease etc.

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