Acid – Base Disorders

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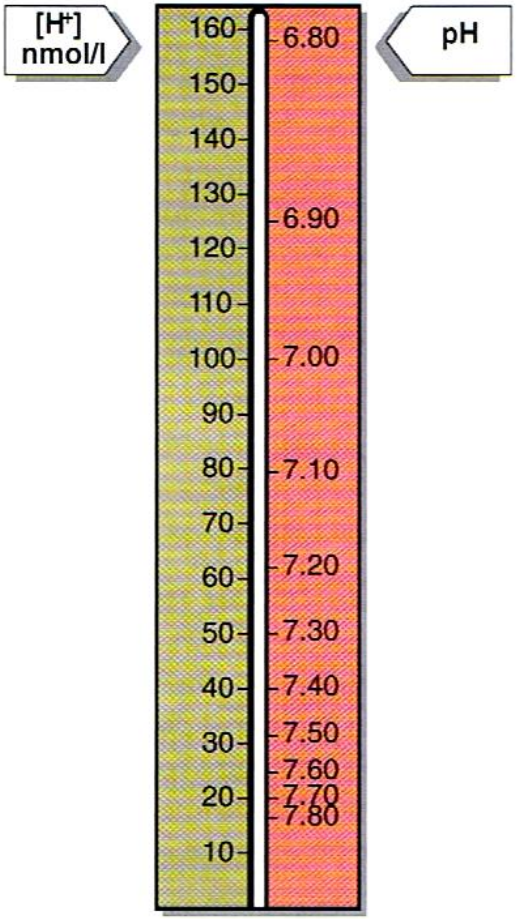
Acid-base balance



- Blood hydrogen ion concentration [H^+] is maintained within tight limits in health. Normal levels lie between 35 - 45 nmol/L (pH 7.35-7.45) in the ECF
- Any H^+ values outside this range will cause alteration in the rates of chemical reactions within the cell and affect many metabolic processes of the body
- Values greater than 120 nmol/L or less than 20 nmol/L are usually incompatible with life

H^+ production

- Hydrogen ions are produced in the body as a result of **metabolism**, (from the oxidation of the sulphur-containing amino acids of protein ingested as food)
- The total amount of H^+ produced each day is 40 – 80 mmol but all the H^+ produced are efficiently excreted in urine
- Everyone who eats a diet rich in protein passes an acidic urine
- Incomplete oxidation of energy substrates generates acid e.g. lactic acid, ketoacids. Further metabolism of these consumes it.
- Temporary imbalances between the rates of production and consumption may arise in health (e.g. accumulation of lactic acid during heavy exercise), but they are in balance and so make no contribution to net hydrogen ion excretion.

- Large amounts of **CO_2** as an acid are produced by cellular activity each day with the potential to upset acid-base balance

- More than 15,000 mmol per 24 h of carbon dioxide is produced in this way

- Although carbon dioxide itself is not an acid, in the presence of water it undego hydration to form a weak acid: carbonic acid

- $CO_2 + H_2O \leftrightarrow H_2CO_3$

- Under normal circumstances all of this CO_2 is excreted via the lungs, having been transported in the blood. Only when respiratory function is impaired do problems occur
- In disease, imbalances between the rates of acid formation and excretion can occur resulting in acidosis or alkalosis.

Buffering and buffers

- A buffer is a solution of the salt of a weak acid that is able to bind H^+. Buffering does not remove H^+ from the body but mop up any excess H^+ produced (as a sponge)

- Buffering is only a short-term solution to the problem of excess H. Ultimately, the body must get rid of the H by renal excretion

- The body contains a number of buffers to correct sudden changes in H production

- Proteins can act as buffers, and the haemoglobin in the erythrocytes has a high capacity for binding H.

Buffers

- In the ECF, bicarbonate buffer is the most important. In this buffer system, bicarbonate (HCO_3^-) combines with H^+ to form carbonic acid (H_2CO_3)
- The association of H with bicarbonate occurs rapidly, but the breakdown of carbonic acid to CO_2 and water happens relatively slowly
- The reaction is accelerated by an enzyme, carbonic anhydrase, which is present particularly in the erythrocytes and in the kidneys

- Only when all the bicarbonate is used up does the system have no further buffering capacity

- The acid-base status of patients is assessed by consideration of the bicarbonate system of plasma

Buffers

- The bicarbonate buffer system is unique in that:
  - The (H_2CO_3) can dissociate to water and CO_2 allowing CO_2 to be eliminated by lung
  - Changes in CO_2 modify the ventilation rate
  - HCO_3^- concentration can be altered by the kidney

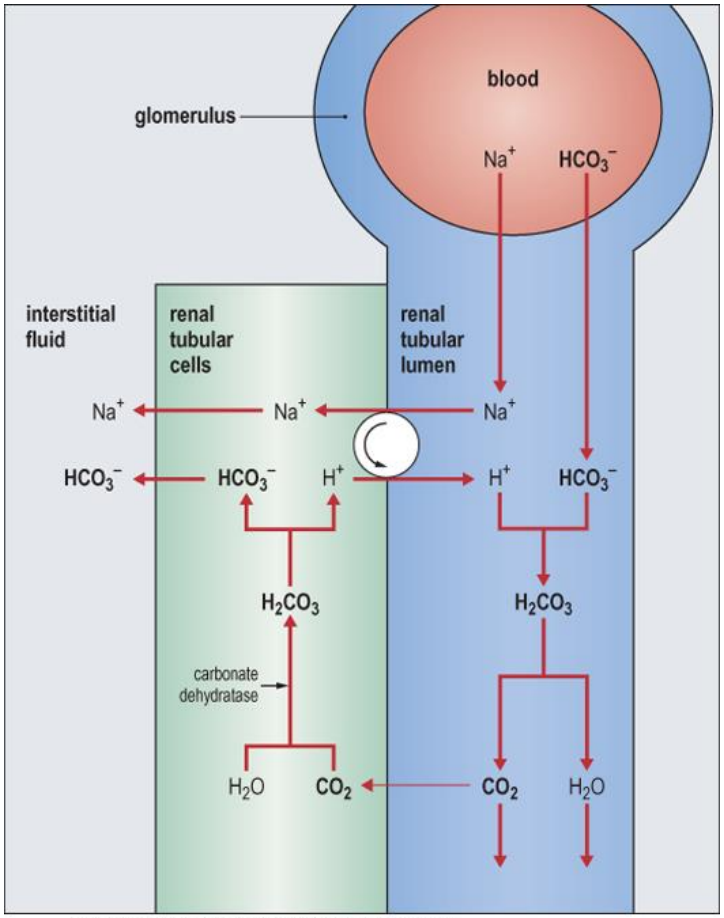
- Phosphate buffer system ($HPO_4^=$ - $H_2PO_4^-$) plays a role in plasma and RBC's and is involved in the exchange of Na/H^+ ion in the urine filtrate

- Plasma proteins, forms important buffer system in plasma. Most circulating proteins has net negative charge capable of H^+ binding

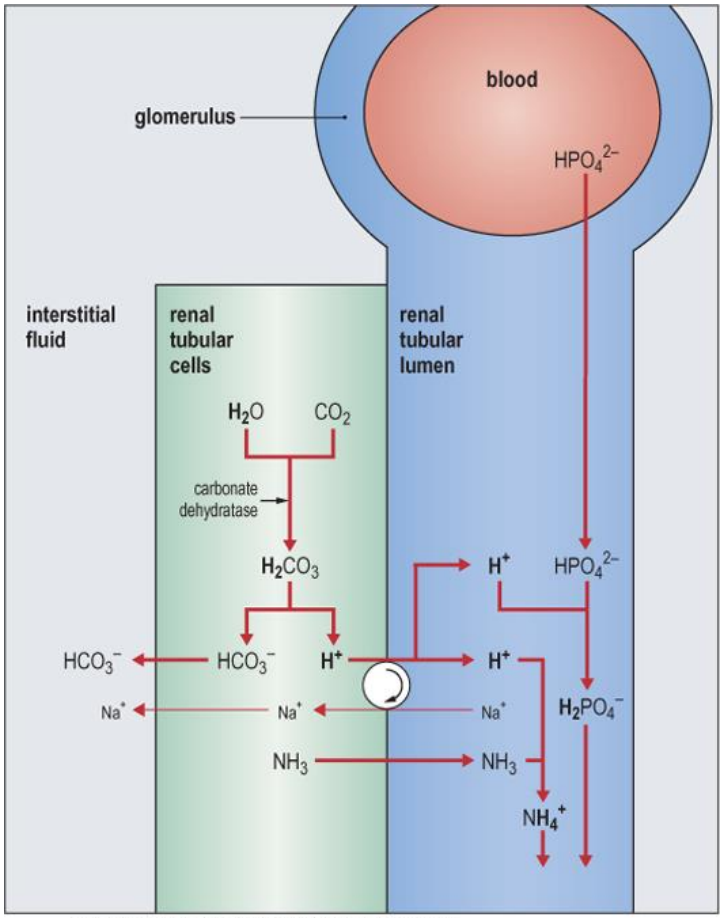
- The proteinaceous matrix of bone is an important buffer in chronic acidosis.

- Ammonia may act as a buffer in renal tubules.

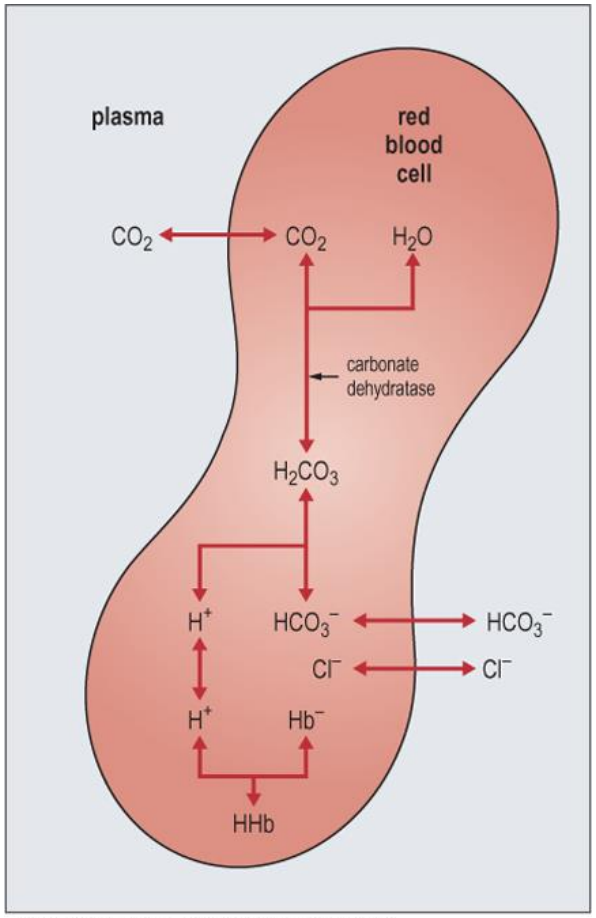
H^+ excretion in the kidney



phosphate & ammonia buffers



Transport of carbon dioxide



Clinical and laboratory assessment of hydrogen ion status

- The carbonic acid (H_2CO_3) component is proportional to the dissolved carbon dioxide, which is in turn proportional to the partial pressure of the CO_2

- Because the body's cellular and metabolic activities are pH dependent, the body tries to restore acid-base homeostasis whenever an imbalance occurs (Compensation)

- the body accomplishes this by altering the factor not primarily affected by the pathologic process. For example, if the imbalance is of non-respiratory origin, the body compensates by altering ventilation (fast response).

- For disturbances of the respiratory component, the kidneys compensate by selectively excreting or reabsorbing anions and cations. The kidneys are slower to respond (2-4 days)

Assessing status

- The [H] concentration in blood varies as the bicarbonate concentration and PCO_2, change, (if every thing else remains constant)
- Adding [H], removing bicarbonate or increasing the PCO_2 will all increase $[H^+]$
- Removing H^+, adding bicarbonate or lowering PCO_2 will all cause the $[H^+]$ to fall.
- An indication of the acid-base status of the patient can be obtained by measuring the components of the bicarbonate buffer system.

Normal ranges

TABLE 14-1. ARTERIAL BLOOD GAS REFERENCE RANGE AT 37°C

pH	7.35–7.45
PCO_2 (mm Hg)	35–45
HCO_3^- (mmol/L)	22–26
Total CO_2 content (mmol/L)	23–27
PO_2 (mm Hg)	80–110
SO_2 (%)	>95
O_2Hb (%)	>95

Causes of metabolic acidosis

- Renal disease. Hydrogen ions are retained .
- Diabetic ketoacidosis. Altered metabolism of fatty acids, as a consequence of the lack of insulin.causes endogenous production of acetoacetic and β –hydroxybutyric acids
- lactic acidosis. particularly tissue anoxia. In acute hypoxic states such as respiratory failure or cardiac arrest. It can be caused by liver disease. The presence of a lactic acidosis can be confirmed by the measurement of plasma lactate concentration.
- Certain cases of overdosage or poisoning. As in salicylate overdose where build-up of lactate occurs, or methanol poisoning when formate accumulates, or ethylene glycol poisoning where oxalate is formed.

The anion gap

- The difference between the sums of the concentrations of the principal cations (Na & K) and principal anions (chloride & bicarbonate) is known as “anion gap”

- anion gap = $(Na+K) – (Cl+HCO_3)$

Causes of lactic acidosis

Causes of lactic acidosis

Tissue hypoxia
decreased perfusion
reduced arterial PO_2

Drugs etc.
ethanol, methanol
phenformin
fructose, sorbitol

Congenital
glucose 6-phosphatase deficiency
other inherited diseases with defective gluconeogenesis or pyruvate oxidation

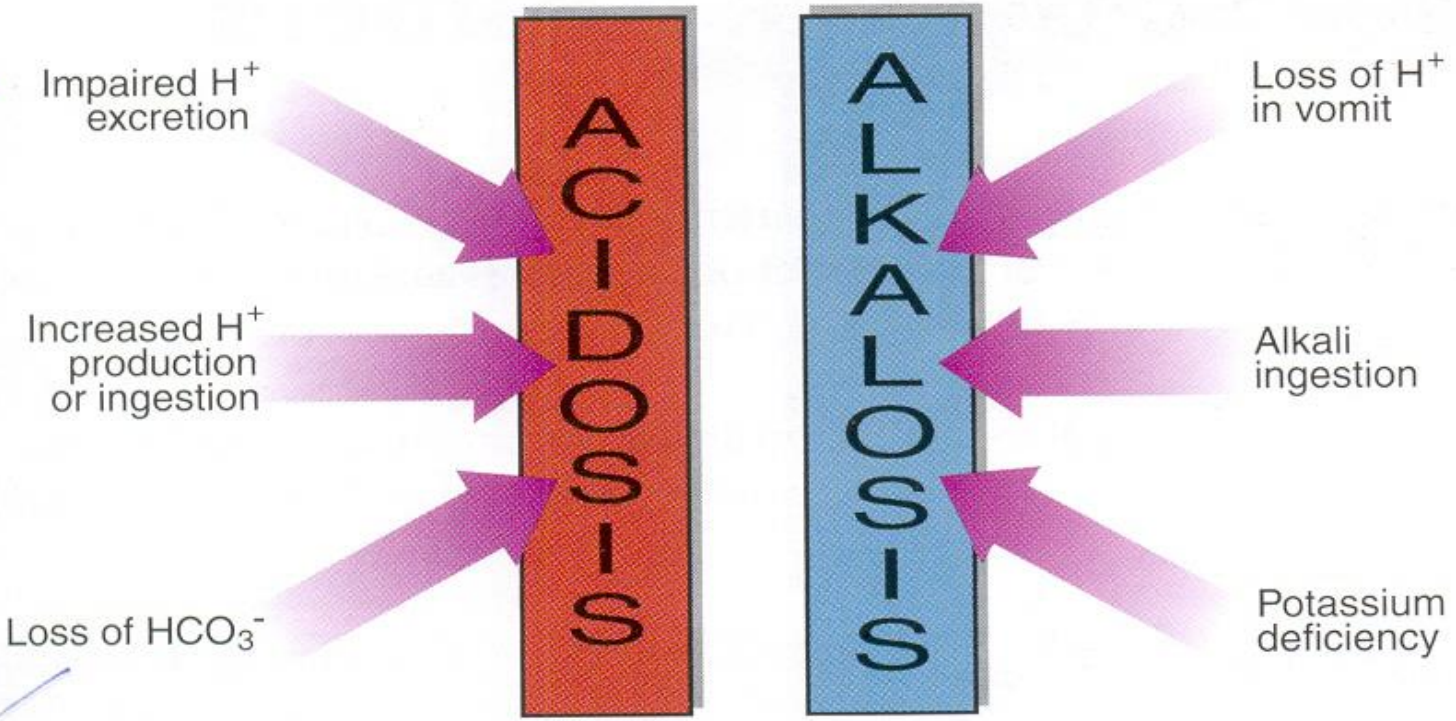


Fig. 3 **Reasons for metabolic acidosis and alkalosis.**

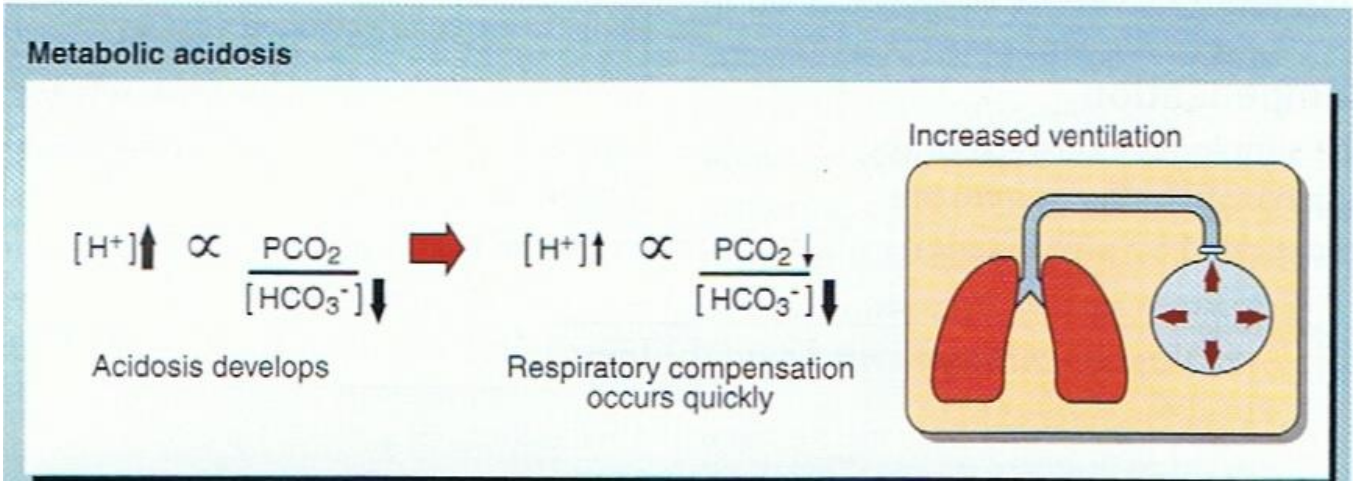
– *Renal tubular acidosis.* Renal tubular

potassium ions. In the renal tubule

Causes of metabolic acidosis

- Metabolic acidosis with a normal anion gap is sometimes referred to as a hyperchloraemic acidosis because a reduced HCO_3 concentration is balanced by increased Cl concentration. It is seen in Chronic diarrhoea or intestinal fistula. Fluids containing bicarbonate are lost from the body

- Renal tubular acidosis. Renal tubular cells are unable to excrete hydrogen ion efficiently and bicarbonate is lost in the urine



Causes of metabolic acidosis

Principal causes of non-respiratory acidosis

Increased H^+ formation
ketoacidosis (usually diabetic, also alcoholic)
lactic acidosis
poisoning, e.g. ethanol, methanol, ethylene glycol and salicylate
inherited organic acidoses

Acid ingestion
acid poisoning
excessive parenteral administration of amino acids, e.g. arginine, lysine and histidine[a]

Decreased H^+ excretion
renal tubular acidoses (types 1 and 4)[a]
generalized renal failure[a]
carbonate dehydratase inhibitors[a]

Loss of bicarbonate
diarrhoea[a]
ileostomy[a]
pancreatic, intestinal and biliary fistulae or drainage[a]
renal tubular acidosis (type 2)[a]

Clinical effects of acidosis

- The compensatory response to metabolic acidosis is hyperventilation. since the increased [H^+] acts as a powerful stimulant of the respiratory centre.

- Hyperventilation is the appropriate physiological response to acidosis and it occurs rapidly

- A raised [H^+] leads to increased neuromuscular irritability. There is a hazard of arrhythmias progressing to cardiac arrest, and this is made more likely by the presence of hyperkalemia, which will accompany the acidosis.

- Depression of consciousness can progress to coma and death.

Metabolic alkalosis

The causes of a metabolic alkalosis may be due to:

- Loss of hydrogen ion in gastric fluid during vomiting. This especially seen when there is pyloric stenosis preventing parallel loss of bicarbonate-rich secretions from the duodenum

- Ingestion of absorbable alkali such as sodium bicarbonate. Very large doses are required to cause a metabolic alkalosis unless there is renal impairment

- Potassium deficiency. In severe potassium depletion. as a consequence of diuretic therapy, hydrogen ions are retained inside cells to replace the missing potassium ions. In the renal tubule more hydrogen ions, rather than potassium, are exchanged for reabsorbed sodium. So, despite there an alkalosis, the patient passes an acid urine.

Causes of metabolic alkalosis

Principal causes of non-respiratory alkalosis

Primarily related to volume depletion/ hypochloraemia

gastrointestinal
- gastric aspiration
- vomiting with pyloric stenosis
- congenital chloride-losing diarrhoea

renal
- diuretic therapy (not K^+-sparing)

Primarily related to potassium depletion

inadequate intake

increased excretion

mineralocorticoid excess
- Conn's syndrome (primary aldosteronism)
- Cushing's syndrome
- Barrter's syndrome and related conditions (see Figs 2.18 and 14.15)

secondary aldosteronism

drugs increasing mineralocorticoid activity,
- carbenoxolone

Administration of alkali

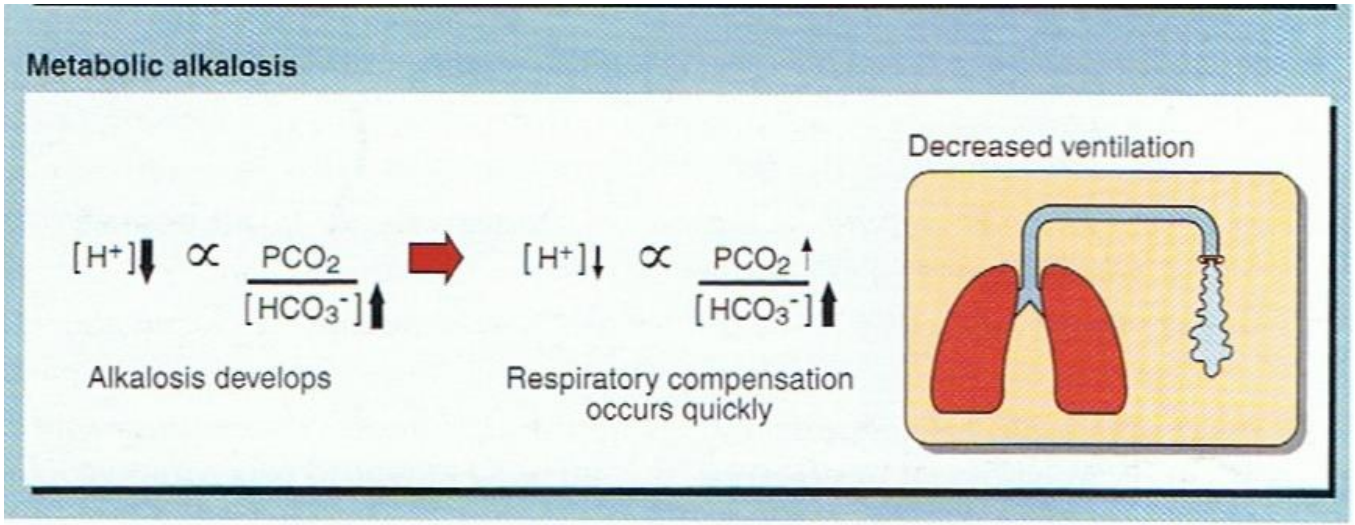
inappropriate treatment of acidotic states

chronic alkali ingestion

Marshall & Bangert: Clinical Chemistry, 6th Edition.

Clinical effects of alkalosis

- The clinical effects of alkalosis include:
  - Hypoventilation
  - Confusion and eventually coma
  - Muscle cramps, tetany and paraesthesia may be a consequence of a decrease in the unbound plasma calcium concentration. which is a consequence of the alkalosis.



Respiratory acidosis

- lung diseases: in which CO_2 is not effectively removed from the blood. In certain patients with chronic obstructive pulmonary disease (COPD), where CO_2 is retained in the blood, causing chronic hypercardia (elevated PCO_2)
- In bronchopneumonia, gas exchange is impaired because of the secretions, white blood cells, bacteria, and fibrin in the alveoli.
- Hypoventilation caused by drugs such as barbiturates, morphine, or alcohol will increase blood PCO_2 levels
- Mechanical obstruction or asphyxiation (strangulation or aspiration).
- Decreased cardiac output, such as in CHF, also will result in less blood to the lungs for gas exchange and an elevated PCO_2
- Kidney will compensate for acidosis but it takes time

Causes of respiratory acidosis

Principal causes of respiratory acidosis

Airway obstruction
chronic obstructive airway disease, e.g.
bronchitis, emphysema
bronchospasm, e.g. in asthma
aspiration

Depression of respiratory centre
anaesthetics
sedatives
cerebral trauma
tumours

Neuromuscular disease
poliomyelitis
Guillain–Barré syndrome
motor neuron disease
tetanus, botulism
neurotoxins, curare

Pulmonary disease
pulmonary fibrosis
severe pneumonia
respiratory distress syndrome

Extrapulmonary thoracic disease
flail chest
severe kyphoscoliosis

Respiratory alkalosis

- The causes include:
  - Hypoxemia
  - Chemical stimulation of the respiratory center by drugs, such as salicylates
  - An increase in the environmental temperature; fever; hysteria (hyperventilation); pulmonary emboli; and pulmonary fibrosis.
- The kidneys compensate by excreting HCO_3- in the urine and reclaiming H^+ to the blood.
- The popular treatment for hysterical hyperventilation, breathing into a paper bag, is self-explanatory.

Causes of respiratory alkalosis

Principal causes of respiratory alkalosis

Increased respiratory drive secondary to hypoxia

high altitude
severe anaemia
pulmonary disease, e.g. pulmonary embolism, pulmonary oedema

Other causes of increased respiratory drive

cerebral disturbances, e.g. trauma, infection and tumours
respiratory stimulants, e.g. salicylates
hepatic failure
Gram-negative septicaemia
primary hyperventilation syndrome
voluntary hyperventilation

Mechanical overventilation

Specimen

- Arterial Blood Specimens is an excellent reference.
- Peripheral venous samples can be used if pulmonary function or O_2 transport is not being assessed
- Depending on the patient, capillary blood may need to be used to measure pH and PCO_2 Although the correlation with arterial blood is good for pH and PCO_2
- Sources of error in the collection and handling of blood gas specimens include the collection device, form and concentration of heparin, speed of syringe filling, maintenance of the anaerobic environment, mixing of the sample to ensure dissolution and distribution of the heparin anticoagulant, and transport and storage time before analysis

Adrenal Function

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Introduction

- Adrenal Gland: An Overview
  - Produces steroid hormones & neuropeptides essential for life
  - Conditions affect blood pressure & electrolyte balance.
  - Hypofunction is treated with exogenous hormone replacement, hyperfunction with pharmacologic suppression or surgery.

- Embryology and Anatomy
  - Composed of outer adrenal cortex & inner adrenal medulla
  - Pyramid-shaped, located just above & medial to kidneys

The Adrenal Cortex by Zone

- Zona Glomerulosa (G-Zone) Cells (outer 10%)
  - Synthesize mineralocorticoids critical for sodium retention, potassium, & acid–base homeostasis
- Zona Fasciculata (F-Zone) Cells (middle 75%)
  - Synthesize glucocorticoids critical to blood glucose homeostasis & blood pressure
- Zona Reticularis (R-Zone) Cells (inner 10%)
  - Sulfate DHEA to DHEA-S (Androgens)

The adrenal gland

- **Adrenal cortex:**
  - glucocorticoids: cortisol
  - mineralocorticoids: aldosterone
  - androgens: dehydroepiandrosterone(DHEA) and androstendione

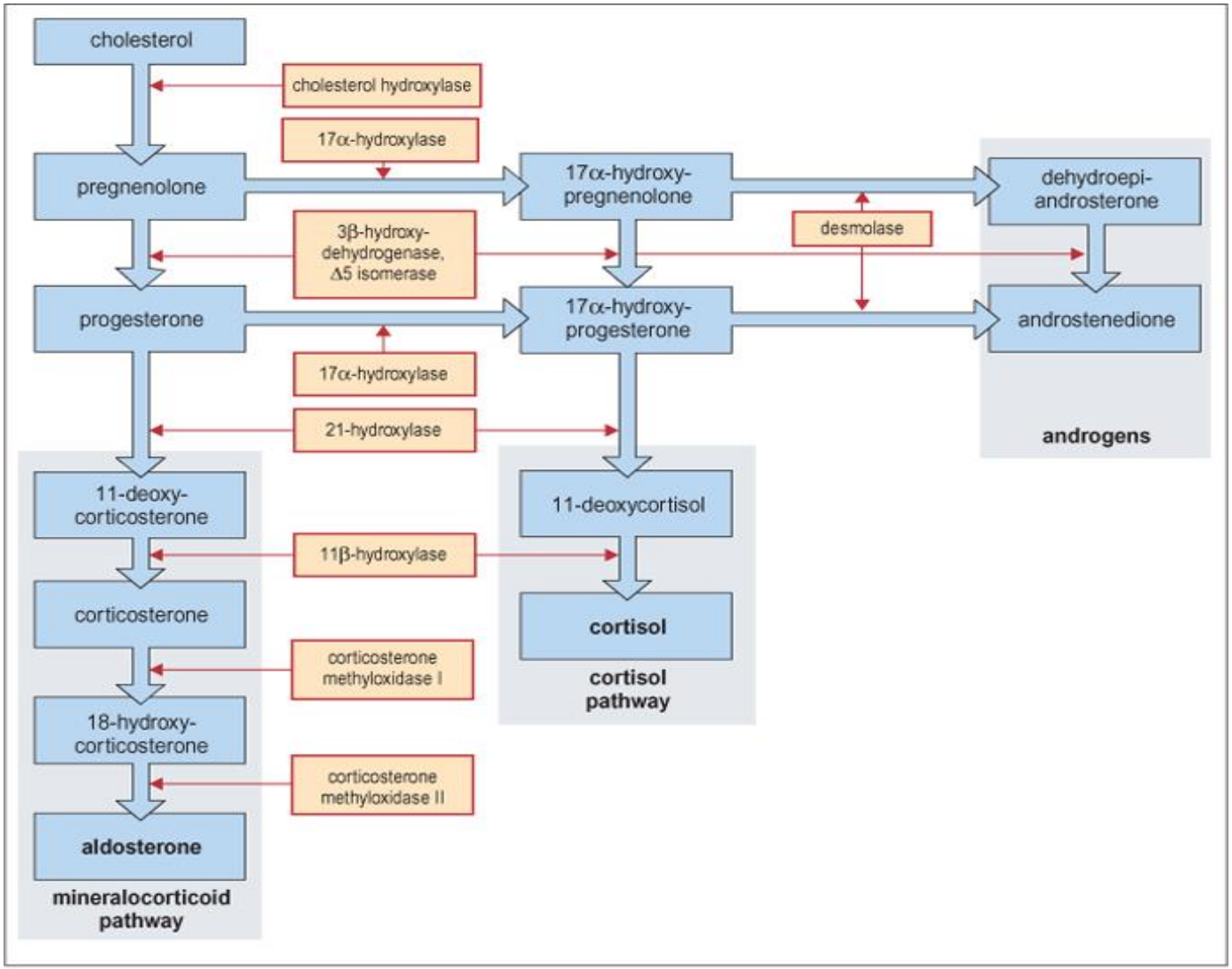
- **Adrenal medulla :**

  Catecholamines:
  - -adrenaline
  - -noradrenaline

The Adrenal Cortex by Zone (cont'd)

- Cortex Steroidogenesis
  - All adrenal steroids are derived by sequential enzymatic conversion of a common substrate, cholesterol.
  - Only free cholesterol can enter steroidogenic pathways in response to ACTH.
  - Conversion of cholesterol to pregnenolone is a rate-limiting step in steroid biosynthesis.
  - Decreased activity of any enzymes required for biosynthesis can occur as an acquired or inherited trait.
  - Evaluation of adrenal function requires measuring relevant adrenal hormones, metabolites, & regulatory secretagogues.

Biosynthesis of adrenal steroid hormones



Actions of glucocorticoids

increase protein catabolism

increase hepatic glycogen synthesis

increase hepatic gluconeogenesis

inhibit ACTH secretion (negative feedback mechanism)

sensitize arterioles to action of noradrenaline (norepinephrine), hence involved in maintenance of blood pressure

permissive effect on water excretion; required for initiation of diuresis in response to water loading

inhibit the inflammatory and immune responses

inhibit bone formation (through inhibition of type 1 collagen synthesis)

Measurement of adrenal steroid hormones

- by specific immunoassays
- Plasma concentrations of these hormones fluctuate for various reasons, so the results of single estimations must be interpreted with caution
- measurement of urinary cortisol is valuable in investigating Cushing's syndrome
- Gas-liquid chromatography analyses of urinary steroids is valuable in the investigation of suspected congenital adrenal hyperplasia (CAH) & adrenal carcinoma

Cortisol

- **Secreted in response to stress**

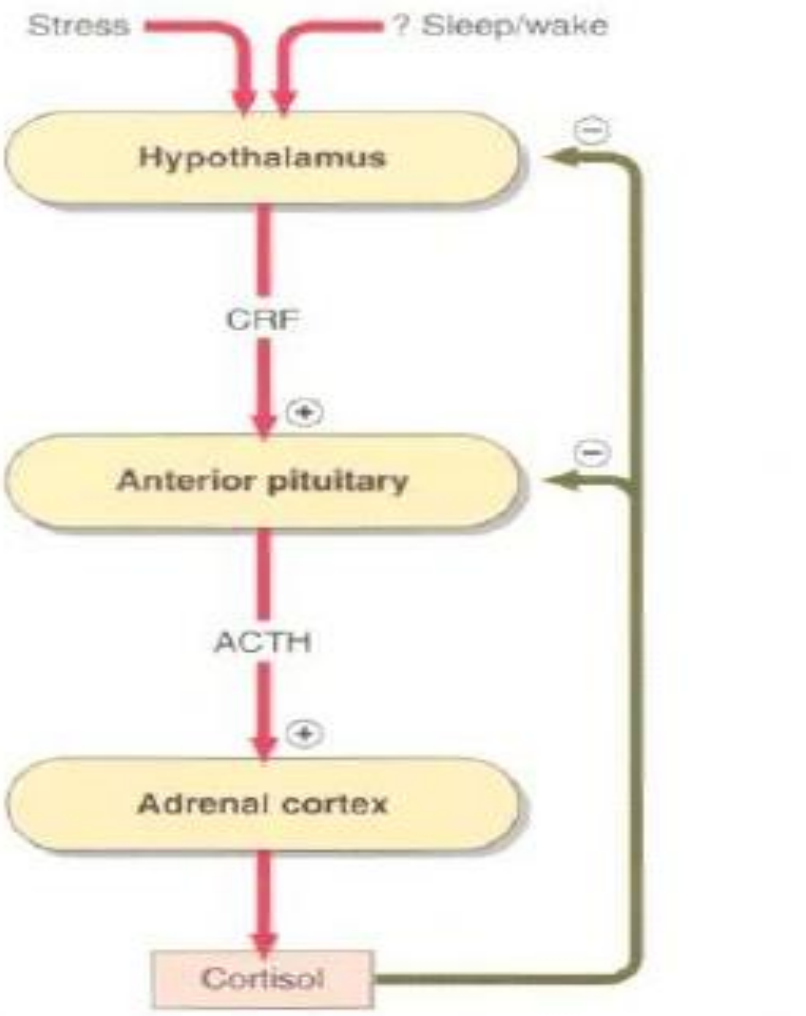
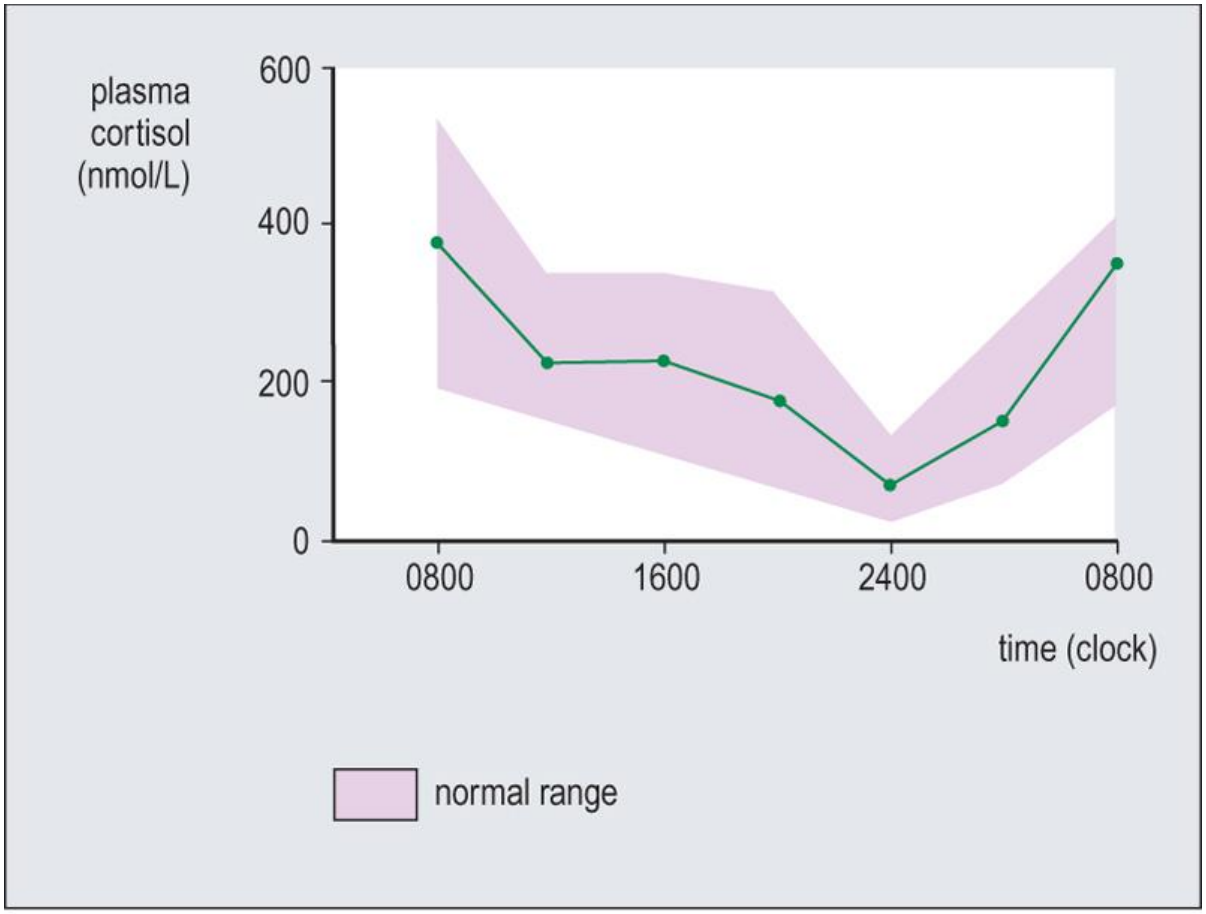


Fig. 2 **The hypothalamic–pituitary–adrenocortical axis.**

Cortisol.. Cont..

- measurement of urinary cortisol is valuable in investigating Cushing's syndrome
- 95%is bound to Corticosteroids Binding Globulin { CBG } (trascortin)
- Trascortin is almost fully saturated at normal cortisol levels
- so:measurement of 24h urinary excretion of cortisol is a sensitive way of detecting increased, but not decreased, secretion of the hormone
- Plasma cortisol show diurnal variation
- Loss of diurnal variation (in blood sample taken an hour before midnight), is an early feature of adrenal hyperfunction(Cushing's syndrome)

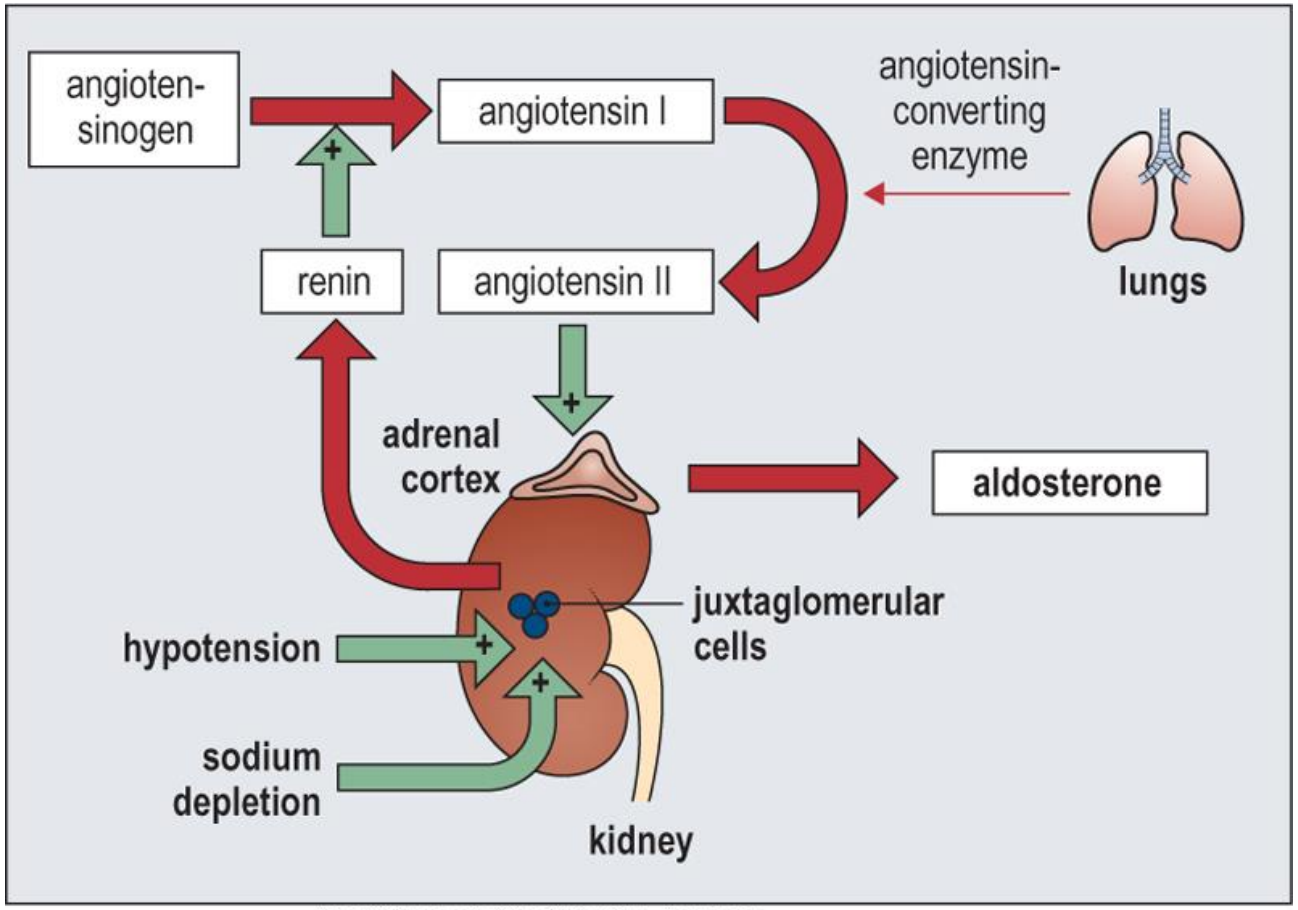
Diurnal variation in plasma cortisol level



Aldosterone

- the most important mineralocorticoid
- Its secretion is stimulated through the action of renin → so measurement of plasma renin activity at the same time of aldosterone level (to establish whether aldosterone secretion is autonomous or under normal control
- posture of patient give variable plasma aldosterone conc.
- aldosterone secretion does not depend upon ACTH
- Secretion of aldosterone is directly stimulated by hyperkalemia

Stimulation of aldosterone secretion



Androgens

- Measurement of androgens is of value in the :
- -diagnosis and management of congenital adrenal hyperplasia (CAH)
- -and in the investigation of virilization in women

Disorders of adrenal cortex

- either:

  * hypofunction….Addison’s disease

or*hyperfunction..Cushing’s syndrome

….Conn’s syndrome

- Both features can be seen in CAH

Adrenal hypofunction (Addison's disease)

- uncommon but life threatening
- increased pigmentation due to high ACTH because of the loss of feed-back mechanism
- adrenal crisis → is a medical emergency (adrenal failure):
*shock, *hypovolemia, and *hypoglycemia
- adrenal failure can occur secondarily to pituitary failure (in such case: abnormal pigmentation does not occur)
- to diagnose whether the failure is primary or secondary, ACTH (synacthen) stimulating test should be performed (short and/or long test)
- plasma ACTH also is measured
  -in primary cases: plasma ACTH is high
  -in secondary cases: plasma ACTH is low

Adrenal hypofunction

Causes	Clinical features
Common	***Common***
glucocorticoid treatment	tiredness, generalized weakness, lethargy
autoimmune adrenalitis	anorexia, nausea, vomiting
tuberculosis	weight loss
	dizziness and postural hypotension
	pigmentation
	loss of body hair (women)
Less common	***Less common***
adrenalectomy	hypoglycaemia
secondary tumour deposits	depression
amyloidosis	
haemochromatosis	
histoplasmosis	
adrenal haemorrhage	

Causes of adrenocortical insufficiency

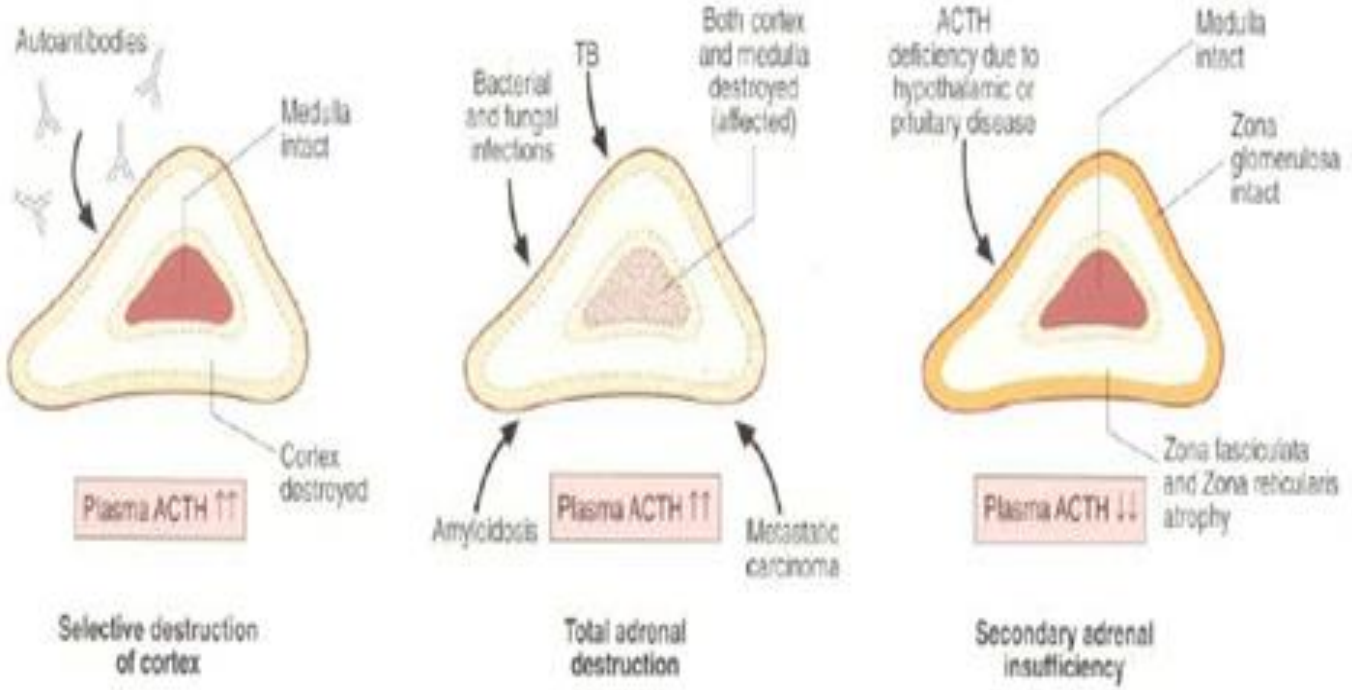


Fig. 2 Causes of adrenocortical insufficiency.

ACTH stimulation tests

Short test	Long test
Procedure take blood sample at 0900 h for measurement of cortisol inject 250 µg ACTH i.m. or i.v. take further blood samples after 30 and 60 min for cortisol measurement	***Procedure*** 0900 h: take blood for measurement of cortisol inject 1 mg depot ACTH i.m. 1500 h: take blood for cortisol measurement 0900 h (next day): take blood for cortisol measurement
Normal results plasma cortisol after ACTH increment of 200 nmol/L with peak of >550 nmol/L	***Results*** primary adrenal insufficiency: no increase in cortisol secondary adrenal insufficiency: increase in cortisol at 6 h with further increase at 24 h; total increment >200 nmol/L

Adrenal hyperfunction

- **Cushin's syndrome:**
  - excessive glucocorticoids (major)
  - excessive mineralocorticoid (minor)
- **Conn's syndrome:**
  - excessive mineralocorticoid

Cushing's Syndrome

- Caused by excess glucocorticoid production or prolonged exogenous steroid use
- Determinations Necessary for Diagnosis
  - ACTH-dependent vs. ACTH-independent hypercortisolism
  - Pituitary vs. ectopic ACTH secretion (determined via high-dose dexamethasone suppression test)
- ACTH Source in Dexamethasone Non-Suppressible patients
  - 60–70% of patients with non-suppressible ACTH secretion to high-dose dexamethasone will have Cushing's disease.

Cushing's syndrome

Causes

corticosteroid or ACTH treatment
pituitary hypersecretion of ACTH (Cushing's disease)
adrenal adenoma
adrenal carcinoma
ectopic ACTH secretion by tumours, e.g. carcinoma of bronchus and carcinoid tumours

Clinical features

truncal obesity ('moon face', buffalo hump, protuberant abdomen)
thinning of skin
purple striae
excessive bruising
hirsutism, especially in adrenal carcinoma
skin pigmentation (only if ACTH elevated)
hypertension
glucose intolerance
muscle weakness and wasting, especially of proximal muscles
menstrual irregularities, hirsutism
back pain (osteoporosis and vertebral collapse)
psychiatric disturbances:
- euphoria
- mania
- depression

Cushing's syndrome

- In addition to the clinical features resulted from cortisol, excessive cortisol have minor mineralocorticoid activity → causing:

Na retention, leading to hypertension,

and potassium wasting, causing a hypokalemic alkalosis

Cushing's.. Cont..

- **Psuedo-Cushing's syndrome:**

-may have some of the biochemical abnormalities of true Cushing's disease.

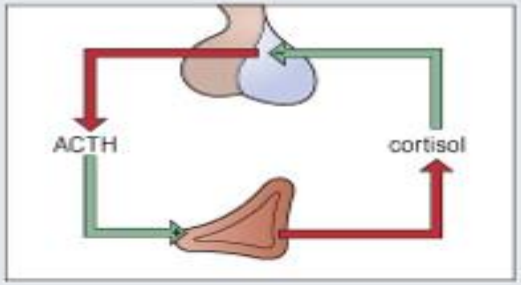
-it can occur in *sever depression and *alcoholics

Cushing's syndrome

a. Normal

production of cortisol by adrenal cortex stimulated by ACTH

cortisol exerts a negative feedback effect on release of ACTH by pituitary

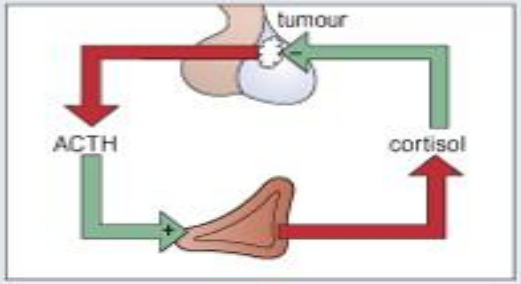


b. Cushing's disease

ACTH secretion increased

pituitary insensitive to feedback by normal levels of cortisol

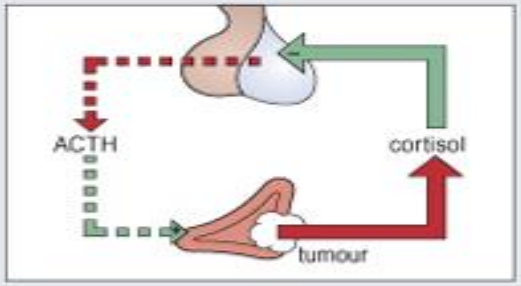
higher levels of cortisol required to produce negative feedback effect on ACTH secretion



c. Adrenal tumours

autonomous cortisol production

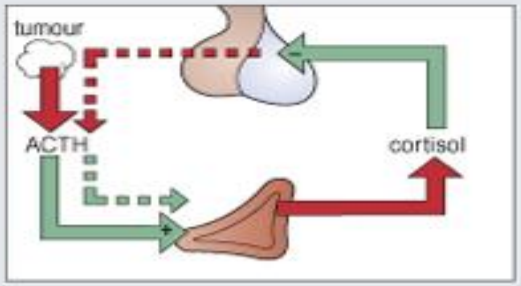
high circulating cortisol inhibits ACTH secretion



d. Ectopic ACTH secretion

high level of ACTH secreted by tumour stimulates excessive cortisol production

inhibition of secretion of ACTH by pituitary



Cushing's Syndrome (cont'd)

- Steps in Diagnosis
  - Document cortisol excess:
    - Urine free cortisol (and/or metabolites)
    - Random plasma cortisol levels
    - Baseline a.m. cortisol concentrations
  - Determine if diurnal rhythm is lost: late-night values remain high.
  - Determine loss of normal cortisol suppression by dexamethasone.

Screening tests for Cushing's syndrome

Test	Normal result
24 h urinary cortisol excretion	<300 nmol/24 h
overnight/48 h low-dose dexamethasone suppression test	plasma cortisol <50 nmol/L at 0900 h

Cushing's Syndrome (cont'd)

- CRH Stimulation Test to Determine ACTH Dependency
  - Distinguishes types of disease (central vs. primary adrenal)
  - CRH is injected, then cortisol & ACTH levels are measured.

Conn's syndrome (primary aldosteronism)

- **excessive production of aldosterone**

Conn's syndrome

Causes

adrenal adenoma[a]
bilateral hypertrophy of zona glomerulosa cells[a]
glucocorticoid-remediable aldosteronism
adrenal carcinoma

Clinical features

hypertension
muscle weakness (occasionally paralysis)
latent tetany and paraesthesiae
polydipsia, polyuria and nocturia

Conn's syndrome.. Cont..

- hypokalemia is a feature.
- hypertension is a consequence to sodium retention
- Increased plasma renin conc. Causes high aldosterone conc.

↓

secondary aldosteronism (more common than primary aldosteronism)

- Measurement of plasma renin activity to distinguish between primary and secondary aldosteronism.

Conditions associated with secondary aldosteronism

Common

congestive cardiac failure
cirrhosis of liver with ascites
nephrotic syndrome

Less common

renal artery stenosis
sodium-losing nephritis
Bartter's and Gitelman's syndromes[a]
renin-secreting tumours

Conn’s syndrome.. Cont..

- Stages of aldosteronism:

*screening:by measurement of aldosterone & renin in plasma(at same time)

*diagnosis:by saline infusion test(Na load should cause inhibition of aldosterone secretion)

*establishment

- Posture :affect plasma aldosterone and renin activity

Congenital adrenal hyperplasia (CAH)

- inherited metabolic disorder of steroid hormones of the adrenal gland
- the defective enzyme in the synthetic pathway determines the type of products
- most of the CAH cases are due to deficiency in **21-hydroxylase** and to less extent **11β-hydroxylase**

↓

- which causes increased ACTH secretion

↓

- this will cause hyperplasia of the gland

↓results in

- accumulation of 17α-hydroxyprogesterone

↓results an

- increased formation of adrenal androgen

In female: at birth
With ambiguous genitalia

in male: pseudo puberty in their 2nd or 3rd year of life

CAH.. Cont..

- but if enzyme deficiency is partial (in females), the conditions appear in early adulthood with hirsutism, amenorrhea or infertility (late onset CAH)

CAH.. Cont..

- diagnosis is made by demonstrating an elevated 17-hydroxyprogesterone

  (17-OHP) in the plasma at least two days after birth

- treatment: by replacement of cortisol and mineralocorticoid, which should suppress the excessive ACTH production and hence

  androgen synthesis

Cont..

- Partial deficiency in 11β-hydroxylase is more common than complete deficiency of the enzyme

↓

- accumulation of 11-deoxycorticosteron (which possess salt retaining property causing hypertension

- diagnosis by demonstration of an increased plasma 11-deoxycortisol or its urinary metabolite

treatment is with cortisol alone: because mineralocorticoid activity is provided by 11-deoxycortisol

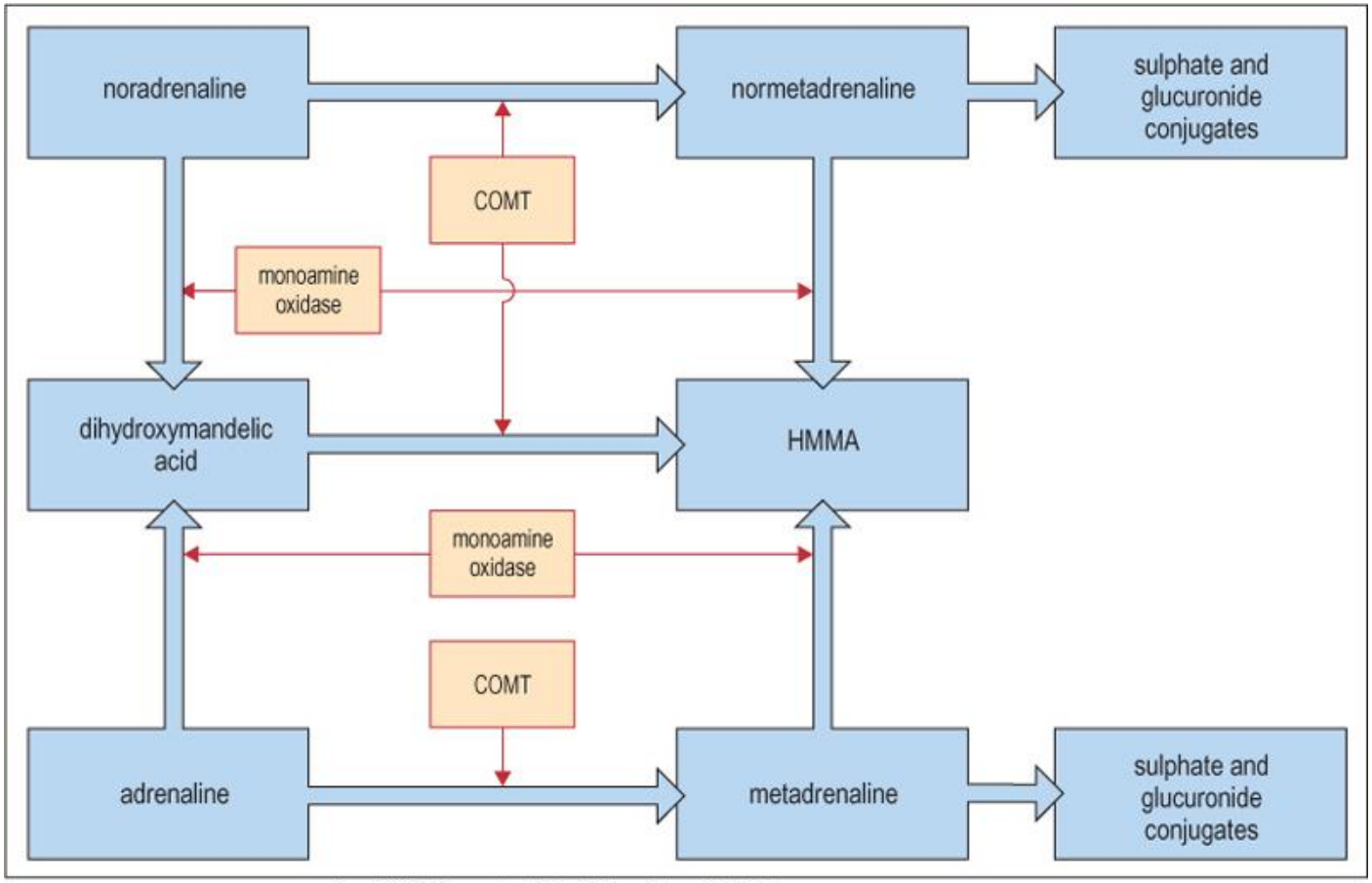
The Adrenal Medulla

- Functions
  - Secretes catecholamines directly into circulation in stead of transmitting messages via efferent axons
  - Acts as first responder to stress (within seconds) to promote fight-or-flight response
- Development
  - Sympathetic cells arise from primordial neural crest stem cells, which differentiate into sympathoblasts or pheochromoblasts.
- Biosynthesis of Catecholamines
  - Norepinephrine & epinephrine synthesized by conversion of phenylalanine substrates in tightly regulated manner

The Adrenal Medulla (cont'd)

- Catecholamine Degradation
  - Three methods of catecholamine elimination: **1)** reuptake into secretory vesicles; **2)** uptake in non-neuronal cells (mostly liver); **3)** degradation
  - Two enzymes (COMT & MAO) produce metabolites from free catecholamines.
  - Metabolites & free catecholamines are filtered into urine & excreted.
- Urine and Plasma Catecholamine Measurements
  - Urine catecholamines are assayed using liquid chromatography, fluorometrics, LC/MS/MS; 24-hour levels are more accurate.

Metabolism of catecholamines



Marshall & Bangert: Clinical Chemistry, 6th Edition.

Disorders of the adrenal medulla

- the main disorder that is of interest to clinical biochemistry is :

  pheochromocytomas

- are tumors that secret catecholamins

The Adrenal Medulla (cont'd)

- Diagnosis of Pheochromocytoma
  - **Fractionated metanephrines & catecholamines** (in a 24-hour collection): best test for diagnosis
  - **Total plasma catecholamines & urine metanephrines:** most sensitive screening profile
  - **Plasma metanephrines** (measured by HPLC or RIA): touted as most specific & sensitive diagnostic test
  - **Urine metanephrines:** possibly most sensitive urine test
  - **Serum chromogranin A & plasma catecholamines**
  - **Clonidine suppression test**
  - **Radiologic localization:** CT, MRI, PET scanning

Calcium, phosphate, and magnesium

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Functions of calcium

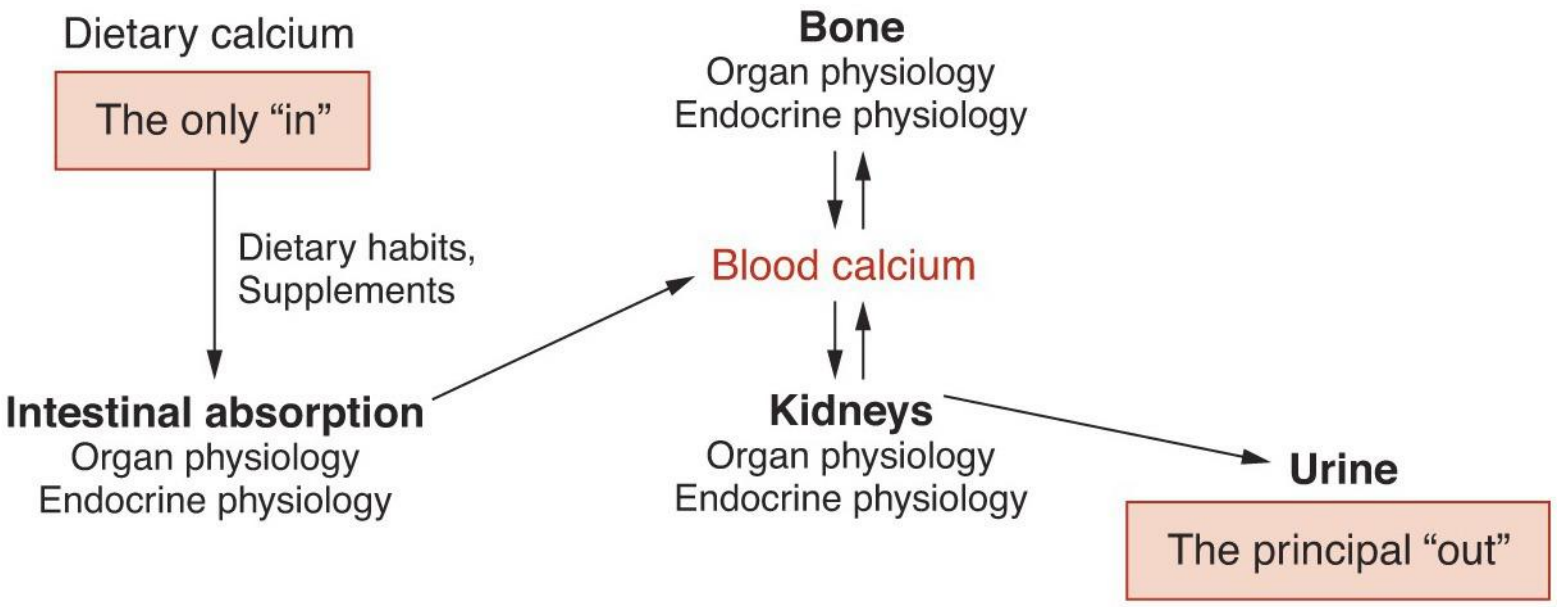
Function	Example
structural	bone teeth
neuromuscular	control of excitability release of neurotransmitters initiation of muscle contraction
enzymic	coenzyme for coagulation factors
signalling	intracellular second messenger

Calcium Homeostasis

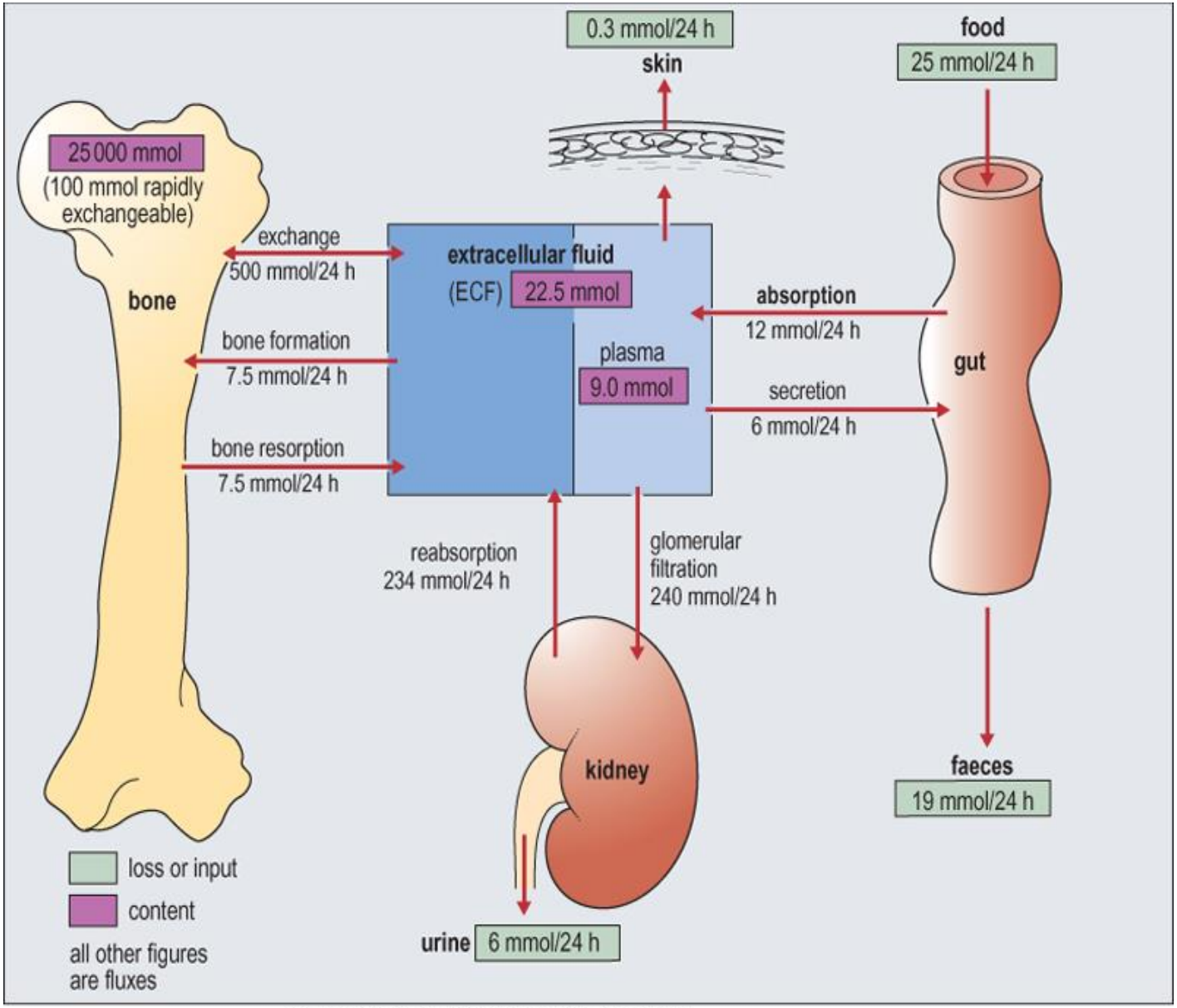
- Cellular & tissue effects of calcium depend on blood calcium maintenance within a specific range.
- Serum calcium maintained at a constant level
- Adult human body contains 1,000 g (1Kg) of calcium
  - 99% as hydroxyapatite salt (bound in the skeleton)
  - 1% in extra-cellular fluids (half in plasma)
  - Diet is only source of calcium
  - Urine is only significant “way out”

Calcium Homeostasis (cont'd)

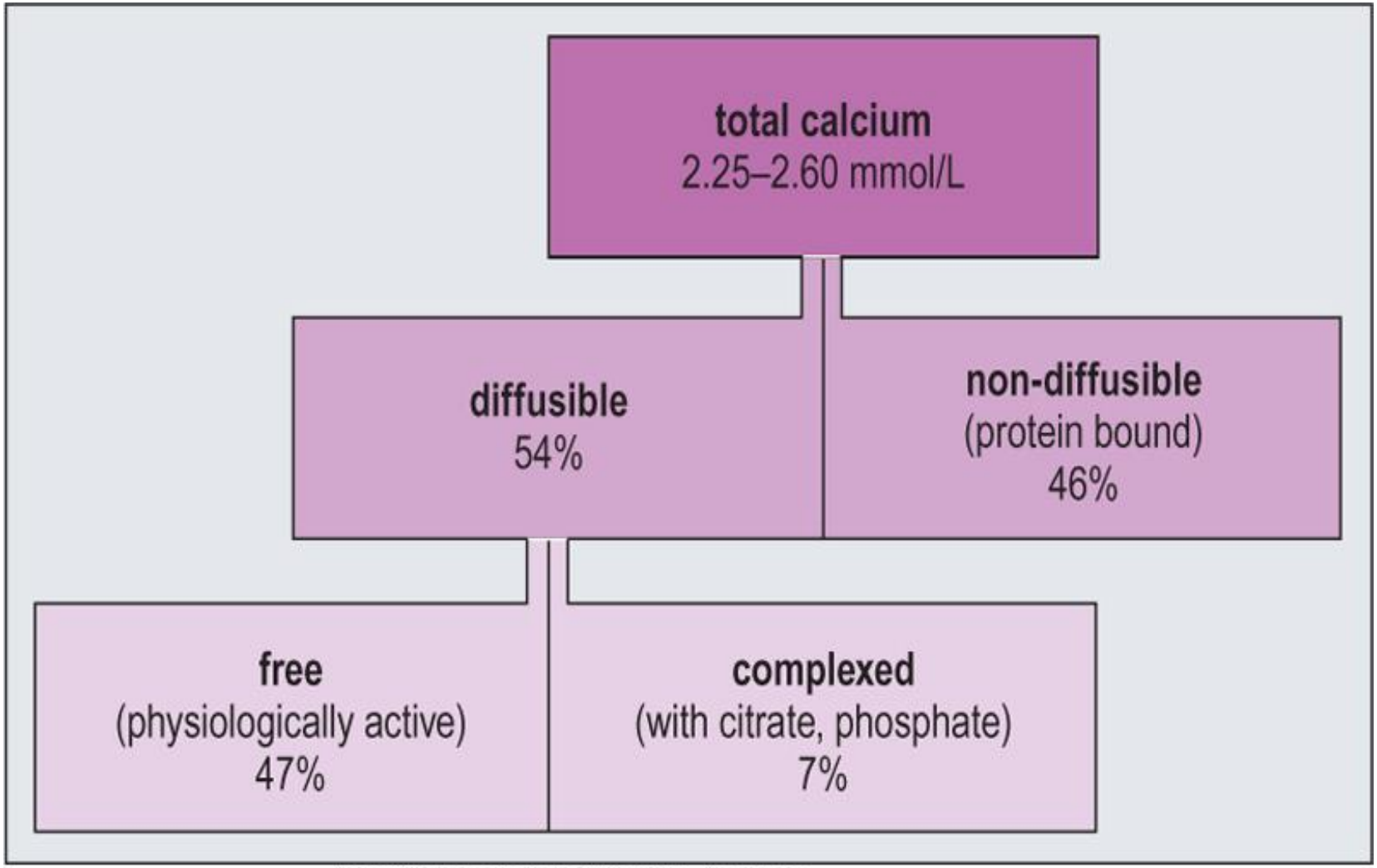
- Calcium homeostasis



Calcium exchange in the body



Distribution of calcium in human plasma



Organ Physiology and Calcium Metabolism

- Bone Physiology
  - **Bone turnover (remodeling):** coupled process of simultaneous bone formation & breakdown occurring throughout life
  - Bone formation is mediated by osteoblasts.
  - Bone breakdown (resorption) is mediated by osteoclasts.
  - When resorption exceeds formation, bone mass decreases (increased risk of fracture).

- Bone always biologically active
- Continuous turnover (remodelling) occurs with bone resorption followed by new bone formation
- At any one time, about 5% of bone mass in adults is subject to remodelling.
  This process is controlled and coordinated by:

  *hormones, *growth factors and *cytokines
- ALP, secreted by osteoblasts, is essential to the process

- Because of the fecal loss, there is a daily dietary requirement of calcium
- This requirement is higher during:

  - growth, pregnancy, and lactation
- Calcium in the ECF pool effectively exchanged through: the kidneys, gut and bone 33 times every 24hours
- A small change in any of these fluxes can have a profound effect on ECF calcium, and hence on plasma calcium

- In alkalosis, calcium binding to albumin increases

↓

the conc. of ionized calcium falls

↓

may produce clinical symptoms and signs of hypocalcemia, although total plasma calcium conc. is unchanged

- In acute acidosis, the reverse effect is observed

↓

the ionized calcium conc. increased

Plasma calcium

- the most frequently used methods for determining plasma calcium conc. is to measure **total calcium**

- measurements of total calcium are satisfactory for most clinical purposes

- Changes in plasma albumin conc. will affect total calcium conc., leading to possible misinterpretation of results in both hypoproteinemic and hyperproteinemic states

Calcium regulating hormones

- in ECF, calcium conc. is normally maintained in narrow limits by a control system of 3 hormones:

  1- parathyroid hormone (PTH)

  2- calcitriol (1,25 dihydroxy cholecalciferol)

  3- calcitonin

- PTH and calcitriol also: control the inorganic phosphate conc. of the ECF

PTH

- secreted by the parathyroid gland in response to a fall in plasma ionized calcium
- Parathyroid glands have calcium-sensing receptors that respond to calcium levels by increasing or decreasing PTH secretion.
- hypercalcemia inhibit PTH secretion
- calcitriol inhibit PTH synthesis
- PTH act on bone and kidney: it increase plasma conc. of calcium, and it decrease the conc. of phosphate
- PTH mobilizes calcium from bone

PTH (cont'd)

- due to increased resorption of bone, so in kidneys: PTH increases filtered amount of calcium

↓

- hypercalciuria is observed despite the increased reabsorption

- also in kidneys: PTH promote phosphaturia by decreasing the reabsorption of filtered phosphate

- it also stimulate calcitriol formation in kidneys

- PTH secretion is magnesium-dependent

Calcitriol

- it is derived from vit D by series of hydroxylations in the liver and kidneys

- in the gut: calcitriol stimulates the absorption of dietary calcium and phosphate

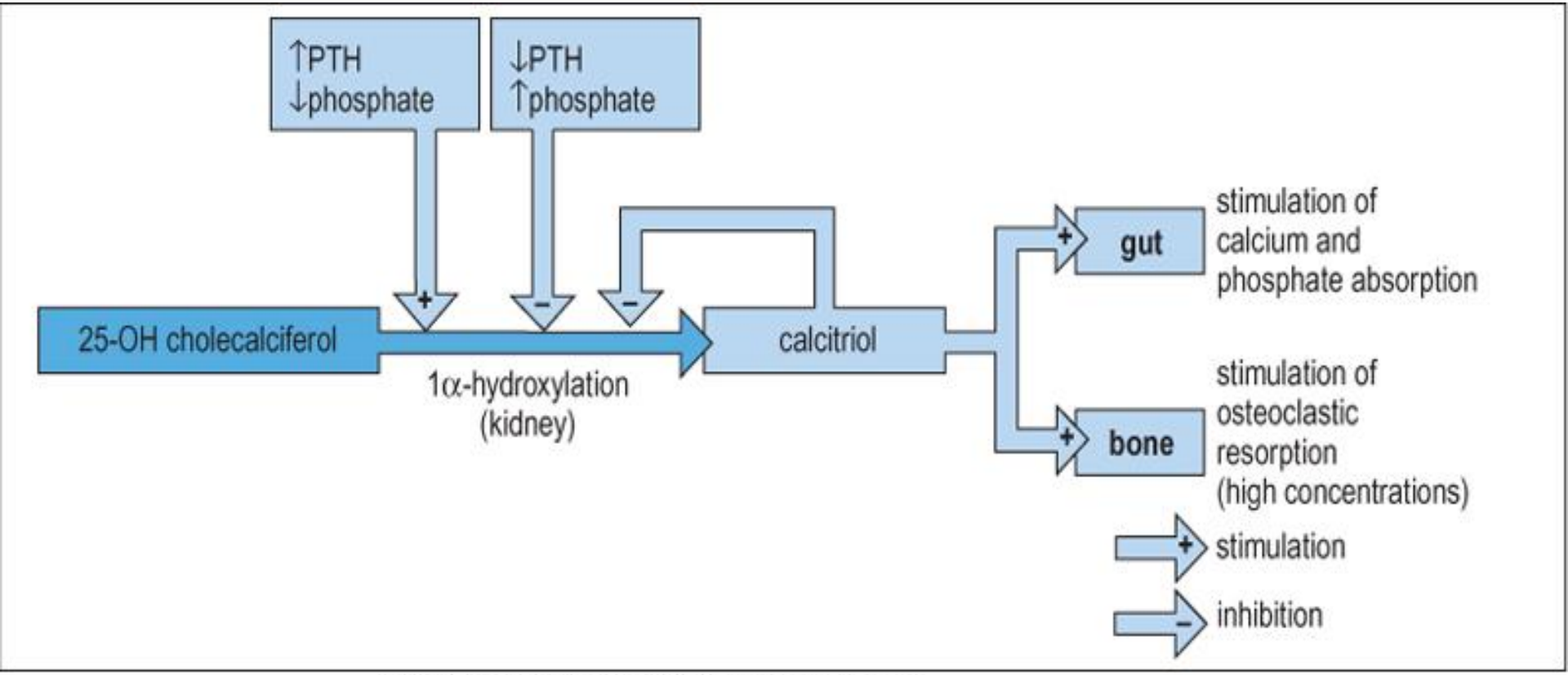
- in bone: calcitriol promote direct mineralization through its role in the maintenance of ECF calcium and phosphate conc.

- at high conc., calcitriol stimulate bone resorption: which release calcium and phosphste into the ECF

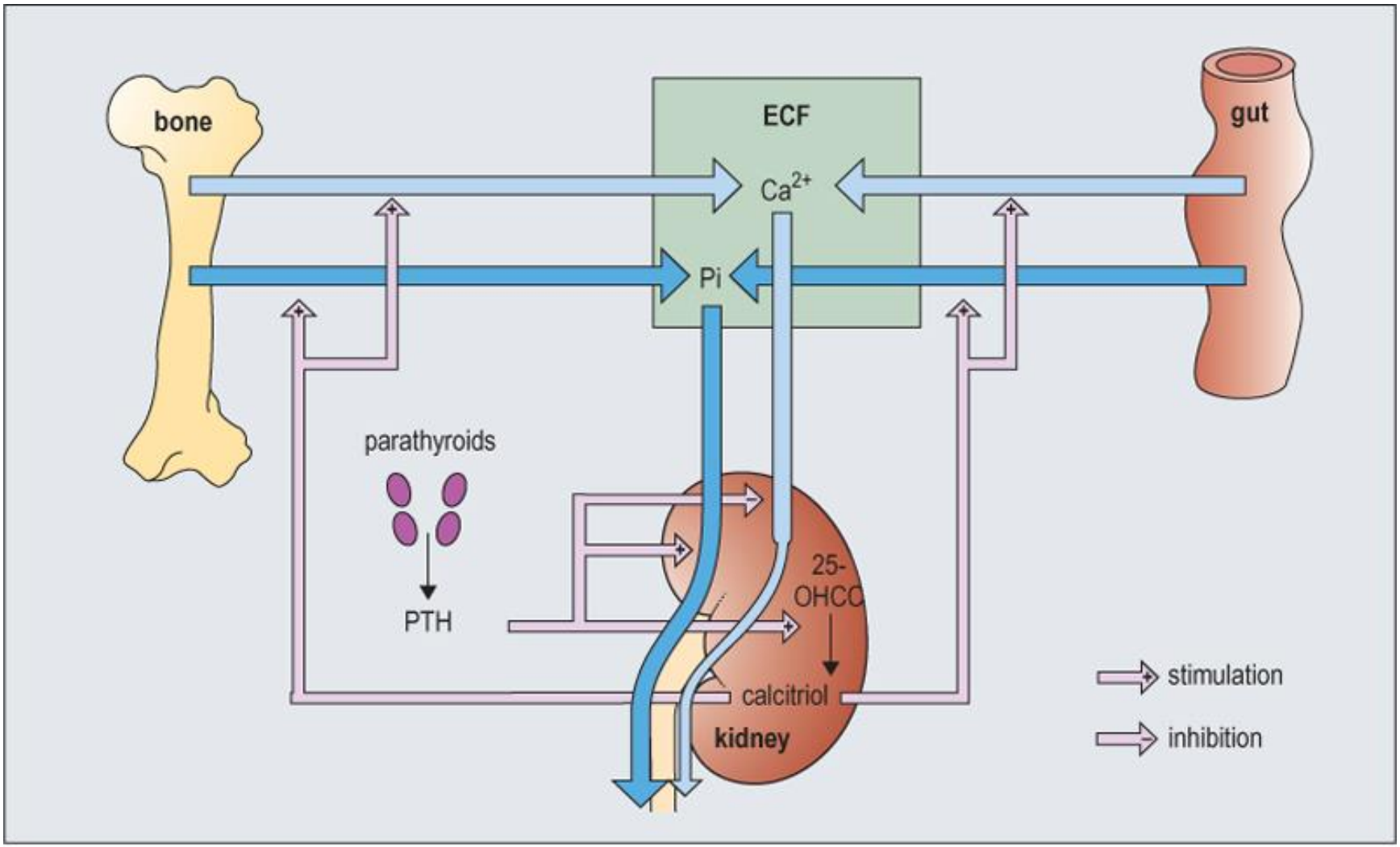
Actions of parathyroid hormone

Target organ	Action	Effect
bone	rapid release of calcium ↑ osteoclastic resorption	↑ plasma [Ca^+]
kidney	↑ calcium reabsorption ↓ phosphate reabsorption ↑ 1α-hydroxylation of 25-hydroxycholecalciferol	↑ plasma [Ca^+] ↓ plasma [Pi] ↑ calcium and phosphate absorption from gut
	↓ bicarbonate reabsorption	acidosis

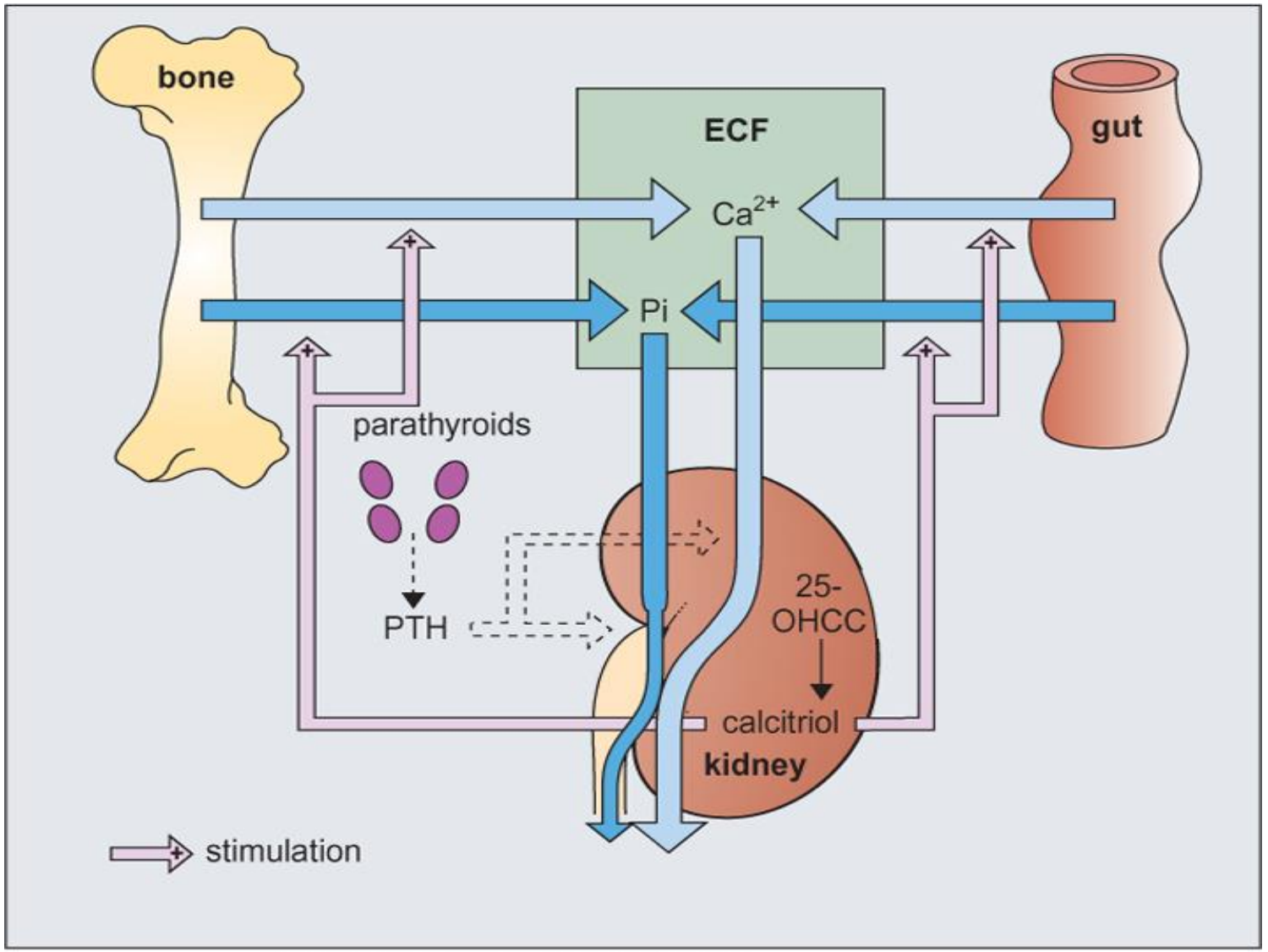
Calcitriol: actions and control of renal synthesis



Homeostatic response to hypocalcemia



Homeostatic responses in hypophosphatemia



Disorders of calcium, phosphate, and magnesium metabolism

Hypercalcaemia

Causes

Common

malignant disease, with or without metastasis to bone
primary hyperparathyroidism

Less common

thyrotoxicosis
vitamin D intoxication
thiazide diuretics
sarcoidosis
familial hypocalciuric hypercalcaemia
renal transplantation (tertiary hyperparathyroidism)

Uncommon

milk–alkali syndrome
lithium treatment
tuberculosis
immobilization (especially in Paget's disease)
acute adrenal failure
idiopathic hypercalcaemia of infancy
diuretic phase of acute renal failure

Clinical features

weakness, tiredness, lassitude, weight loss and muscle weakness
mental changes (impaired concentration, drowsiness, personality changes, coma)
anorexia, nausea, vomiting and constipation
abdominal pain (rarely peptic ulceration and pancreatitis)
polyuria, dehydration and renal failure
renal calculi and nephrocalcinosis (mainly associated with primary hyperparathyroidism)
short QT interval on ECG
cardiac arrhythmias and hypertension
corneal calcification and vascular calcification

there may also be features of the underlying disorder, such as bone pain in malignant disease and hyperparathyroidism

Hypercalcemia

- in hyperparathyroidism and malignant disease, hypercalcemia is associated with low plasma phosphate conc.

(raised plasma phosphate is seen in both cases if there is renal impairment)

- ALP activity is increased

- measurement of PTH is essential

Hypercalcemia (cont'd)

- Endocrine Causes of Hypercalcemia
  - Primary hyperparathyroidism
    - Most common cause of hypercalcemia in outpatient setting
    - Physiologic defect in parathyroid glands themselves
    - Results from adenoma(s) or hyperplasia of parathyroid glands
  - Hypervitaminosis D
    - Caused by excessive intake of vitamin D or aberrant production of $1,25(OH)_2D$
  - Cancers , tumors
    - Cause release of hormones or hormone-like substances

Hypercalcemia (cont'd)

- Familial Hypocalciuric Hypercalcemia
  - Benign condition
  - Germline mutation of CSR (calcium sensing receptor)
  - Stable, mild hypercalcemia from birth
  - Hypocalciuria

Hypocalcemia

Hypocalcaemia

Causes	Clinical features
Artefactual (blood collected into EDTA tube)	behavioural disturbance and stupor numbness and paraesthesiae
Associated with low PTH concentration hypoparathyroidism hypomagnesaemia hungry bone syndrome (see p. 248) neonatal hypocalcaemia	muscle cramps and spasms (tetany) laryngeal stridor convulsions cataracts (chronic hypocalcaemia) basal ganglia calcification (chronic hypocalcaemia) papilloedema Trousseau's sign positive Chvostek's sign positive prolonged QT interval on ECG
Associated with high PTH concentration vitamin D deficiency: dietary malabsorption inadequate exposure to ultraviolet light disordered vitamin D metabolism: renal failure anticonvulsant treatment 1α-hydroxylase deficiency pseudohypoparathyroidism acute pancreatitis high phosphate intake (rare) massive transfusion with citrated blood acute rhabdomyolysis	

Hyperphosphatemia

Some causes of hyperphosphataemia

renal failure
hypoparathyroidism
pseudohypoparathyroidism
acromegaly
excessive phosphate intake/administration
vitamin D intoxication
catabolic states, e.g. tumour lysis syndrome

Hypophosphatemia

Some causes of hypophosphataemia

Redistribution

diabetic ketoacidosis (recovery phase)
enteral/parenteral nutrition with inadequate phosphate (particularly in malnourished patients); intravenous glucose therapy
respiratory alkalosis

Renal loss

primary hyperparathyroidism
renal tubular disease
diuretics
hypophosphataemic rickets
tumour-associated osteomalacia

Decreased intake/absorption

dietary
malabsorption
vomiting
phosphate binding agents, such as magnesium and aluminium salts (rare)
vitamin D deficiency
alcohol withdrawal

Magnesium

- in adult humans, half the body content of magnesium is in bone
  -the rest is divided equally between muscle and other soft tissues

- plasma conc. Being 0.8 – 1.0 mmol/L

- urinary magnesium excretion is increased in:
  *hypercalcemia and *hypermagnesemia
  -and decreased in the oppisite of these states

- PTH and aldosterone: both affect the renal handling of magnesium

- Hypermagnesemia : is uncommon

Hypomagnesemia

Hypomagnesaemia

Causes

malabsorption, malnutrition and fistulae
alcoholism (chronic alcoholism and alcohol withdrawal)
cirrhosis
diuretic therapy (especially loop diuretics)
renal tubular disorders (in advanced renal disease, hypermagnesaemia is usual)
chronic mineralocorticoid excess
drug toxicity, e.g.
- amphotericin
- aminoglycosides
- cisplatin
- ciclosporin

Gitelman's syndrome

Clinical features

tetany (with normal or decreased calcium)
agitation, delirium
ataxia, tremor, choreiform movements and convulsions
muscle weakness, cardiac arrhythmias

Disorders of Hypothalamus and Pituitary Glands

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Introduction

- Pituitary
  - "Master gland": secretes hormones that regulate other glands
  - Needed for metabolism & gonadal, thyroidal, & adrenal function
  - A transponder that translates neural input into a hormonal or endocrinologic product

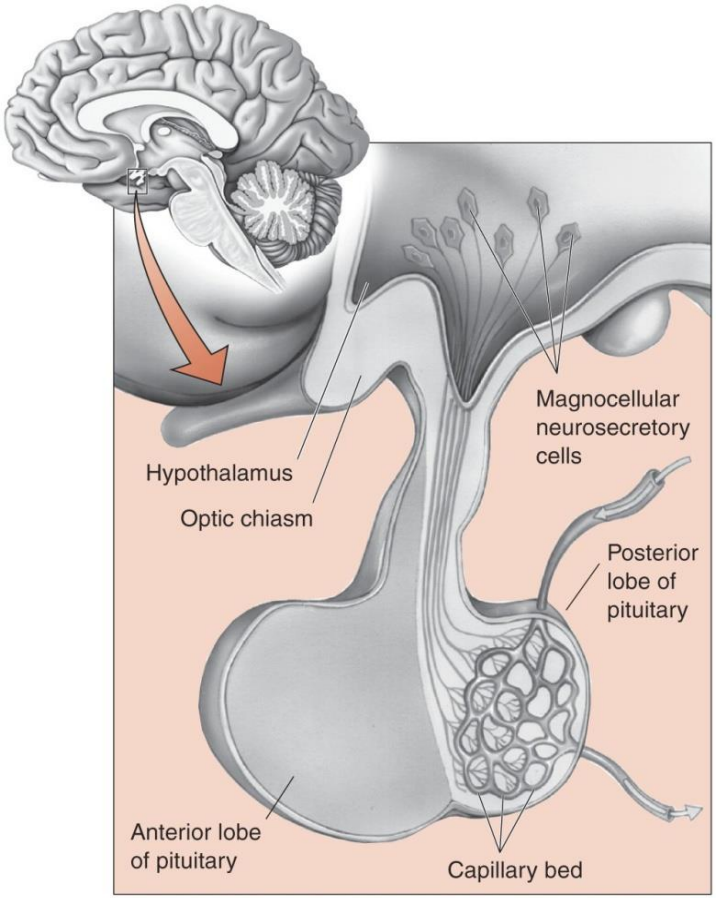
  - Distinguishing features of pituitary function
    - Feedback loops
    - Pulsatile secretions
    - Diurnal rhythms
    - Environmental or external modification of its performance

Embryology and Anatomy

- Three distinct parts of pituitary:
  - 1. Anterior pituitary or adenohypophysis
    - Largest portion of gland; originates from Rathke's pouch
  - 2. Intermediate lobe or pars intermedialis
    - Poorly developed in humans; has little functional capacity
  - 3. Posterior pituitary or neurohypophysis
    - Arises from diencephalon; responsible for storage & release of oxytocin & vasopressin
- Pituitary resides in a pocket of sphenoid & is surrounded by dura mater.

Embryology and Anatomy (cont'd)

- Relational anatomy of pituitary and hypothalamus



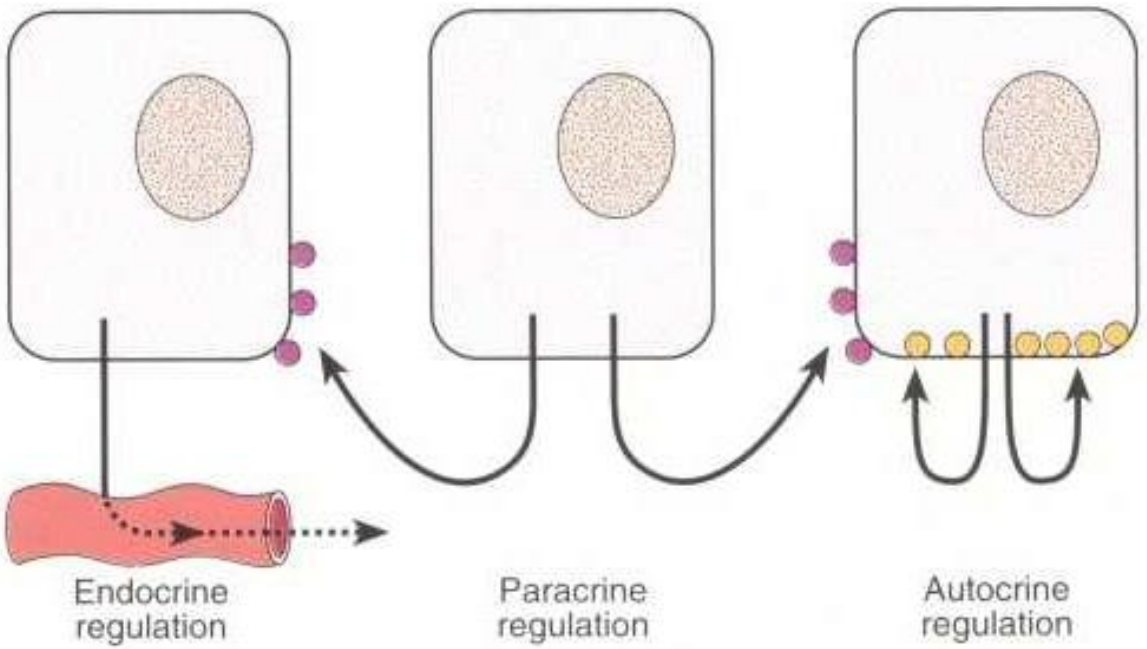


Fig. 1 **Biochemical regulation of cell function.**

Hypothalamic and anterior pituitary hormones

- The hypothalamus secretes a number of hormones or factors that pass down the hypothalamus –hypophyseal portal blood vessels to the pituitary where they regulate the release of hormones from the anterior pituitary.
- The secretion of hypothalamic hormones are Influenced by higher centers in the brain
- The secretion of both hyp. & pit. hormones is regulated by feedback from target hormones.

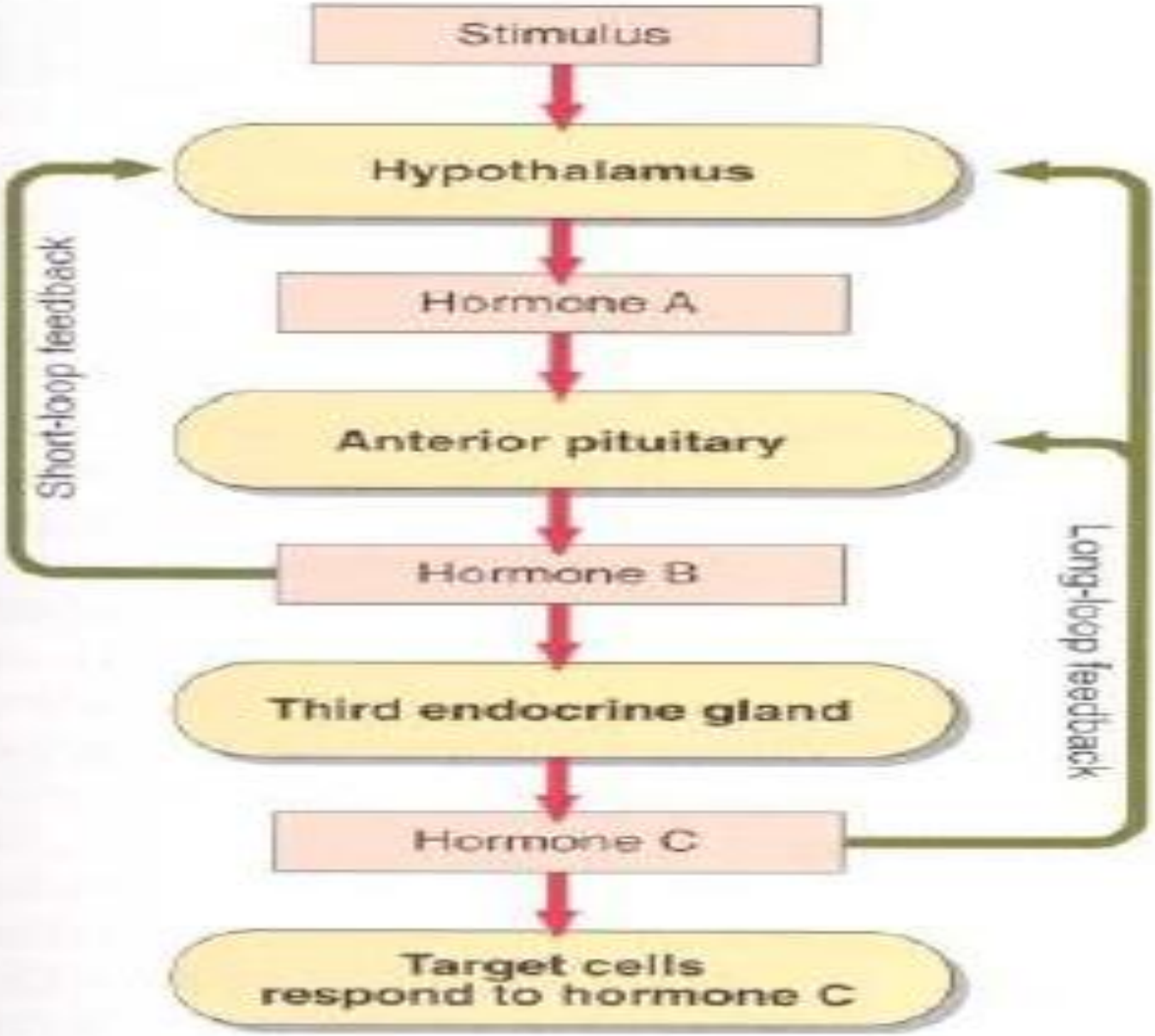
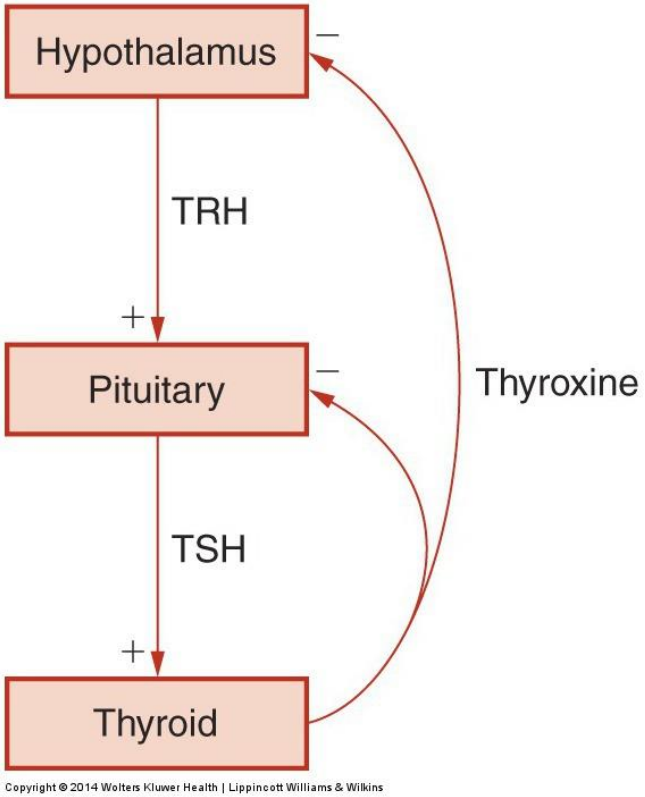
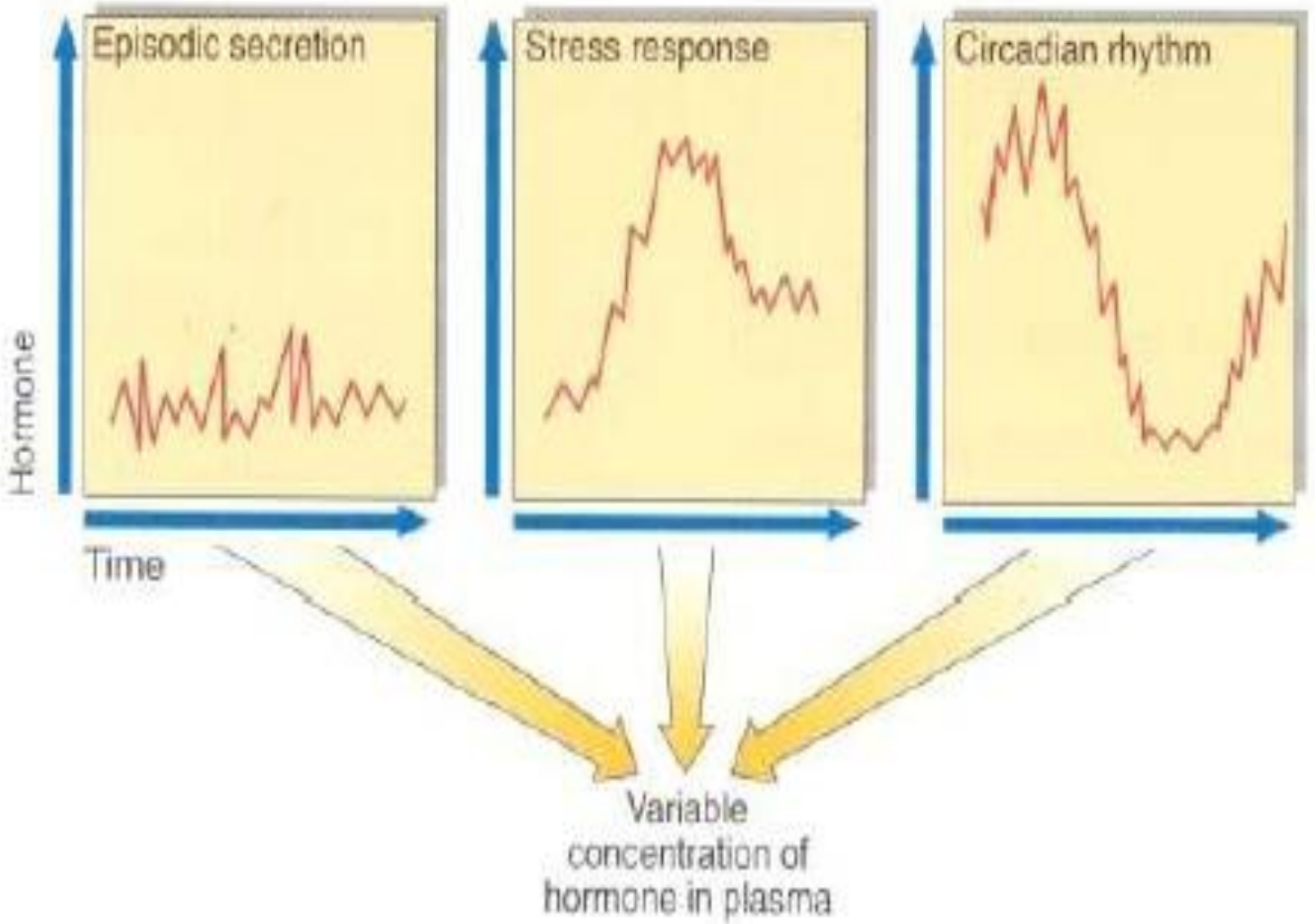


Fig. 2 **Feedback interactions in a hypothalamic-pituitary-endocrine gland system.**

Functional Aspects of the Hypothalamic-Hypophysial Unit

- Simple feedback loop





Reasons why a single blood hormone measurement may have little clinical value.

Anterior pituitary hormones

Anterior pituitary hormones		
Hormone	**Target organ**	**Action**
growth hormone (GH)	liver others	somatomedin synthesis, hence growth stimulation metabolic regulation
prolactin	breast	lactation
thyroid-stimulating hormone (TSH)	thyroid	thyroid hormone synthesis and release
follicle-stimulating hormone (FSH)	ovary testis	oestrogen synthesis oogenesis spermatogenesis
luteinizing hormone (LH)	ovary testis	ovulation corpus luteum, hence progesterone production testosterone synthesis
adrenocorticotrophic hormone (ACTH)	adrenal cortex skin	glucocorticoid synthesis and release pigmentation
β-lipotrophin		precursor of endorphins

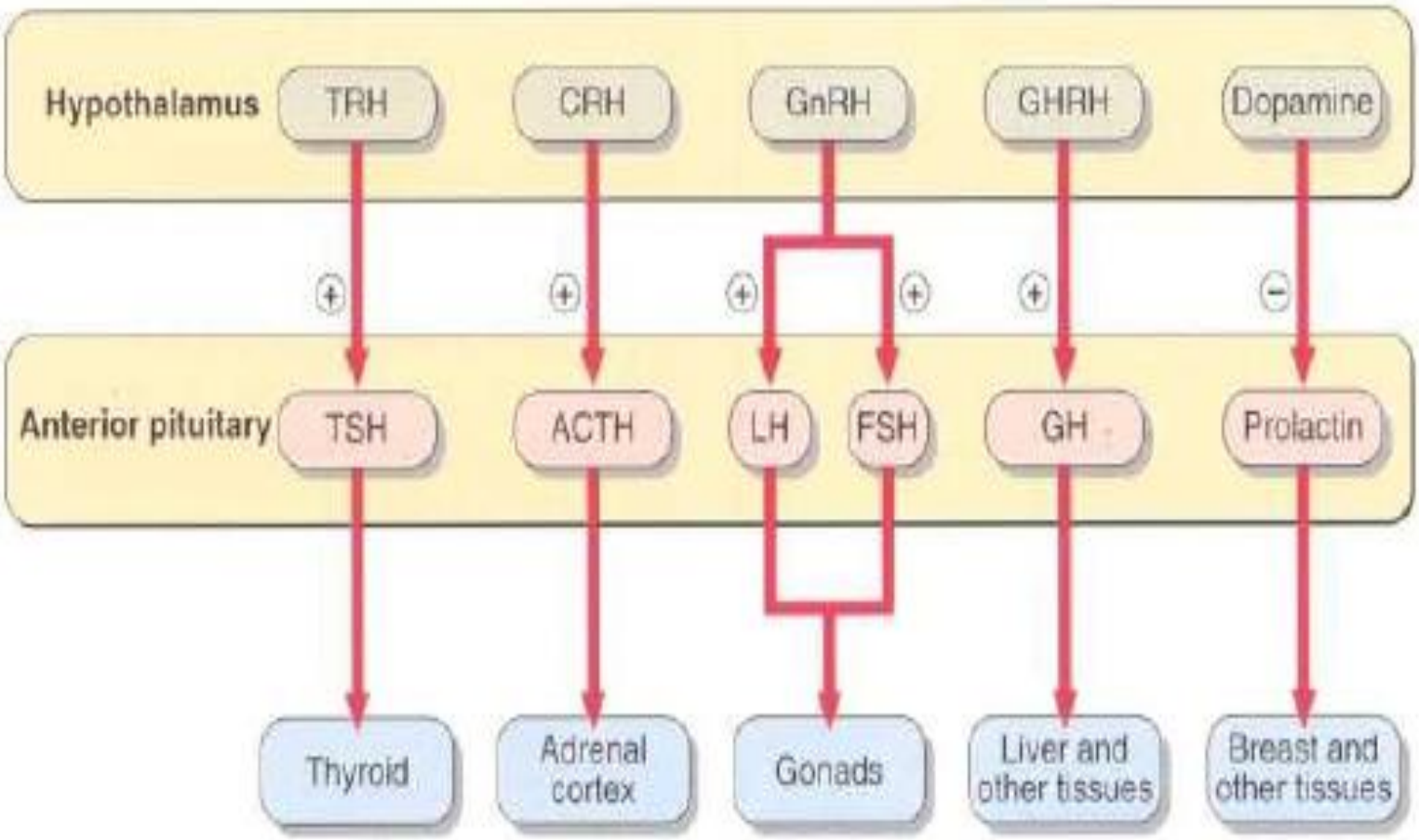


Fig. 1 **Hypothalamic factors which regulate anterior pituitary function.**

Growth hormone

- Polypeptide hormone
- Essential for normal growth
- Stimulate liver to produce IGF-1 (somatomedin-C)
- It has a number of metabolic effects
- Release of GH is controlled by:

  *GHRH
  *somatostatin

  } hypothalamic hormones

- GH secretion is stimulated by:

  ●stress ●exercise ●fasting

  ●a fall in blood glucose conc. ● ingestion of certain amino acids

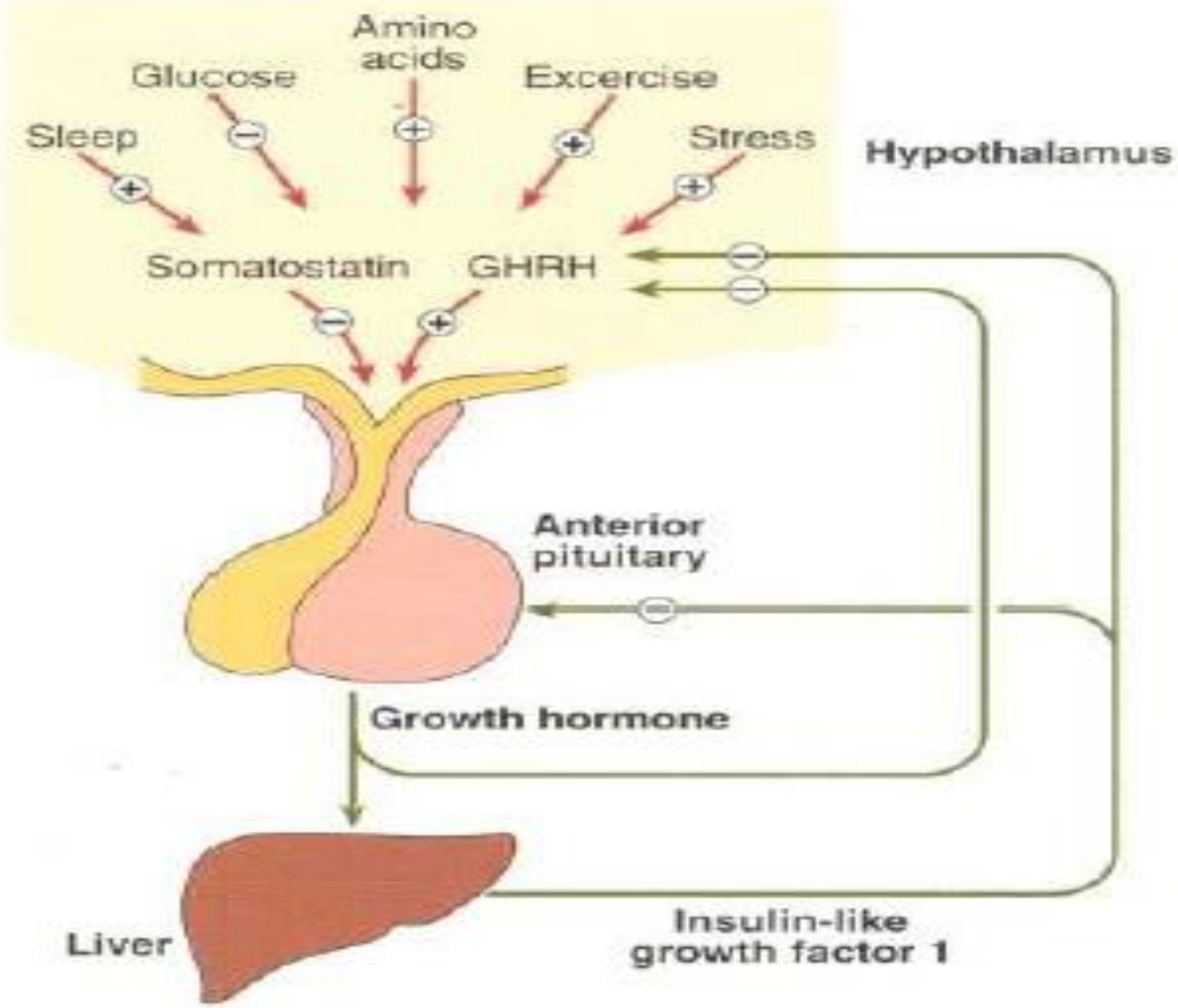


Fig. 2 **The normal regulation of GH secretion.**

Metabolic actions of growth hormone

increases lipolysis (hence ketogenic)

increases hepatic glucose production and decreases tissue glucose uptake (hence diabetogenic)

increases protein synthesis (hence anabolic)

Growth Hormone (cont'd)

- Testing
  - Based on dynamic physiology of growth hormone axis
  - Testing for autonomous production of growth hormone relies on normal suppressibility of growth hormone by oral glucose loading.
  - Strategies to stimulate growth hormone
    - Insulin-induced hypoglycemia (outdated method)
    - Combination infusions of GHRH & amino acid L-arginine
    - Infusion of L-arginine coupled with oral L-DOPA

GH (cont'd)

- GTT is used in the diagnosis of excessive GH secretion (because glucose rise in blood inhibit GH secretion)
- Excessive secretion (pituitary tumor) → causes

  *gigantism in children

  *acromegaly in adults
- Deficiency causes :
- In children→ growth retardation
- In adults→ multiple effects

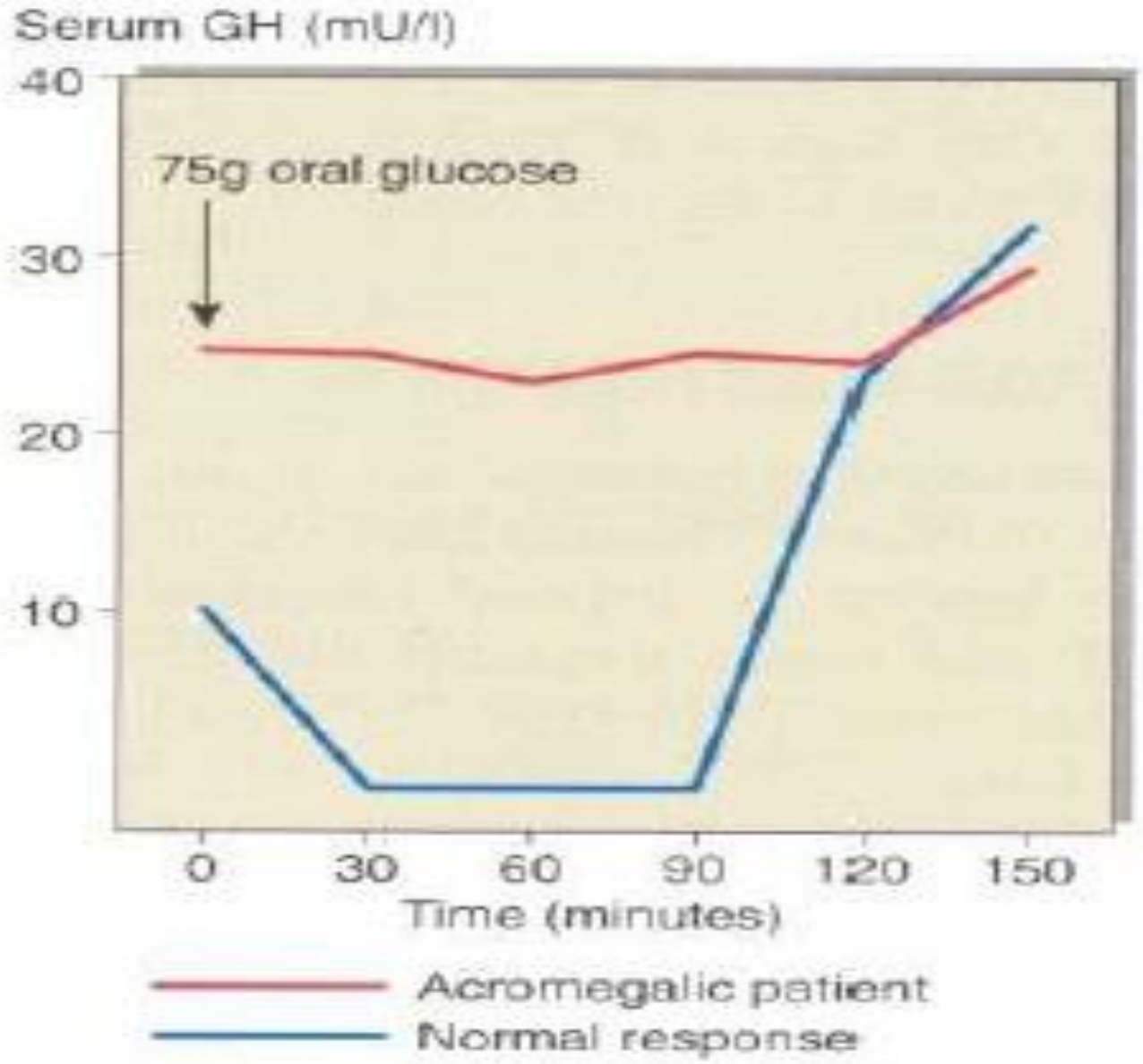


Fig. 4 **The response of GH in a glucose tolerance test in a normal and acromegalic patient.**

Fig. 3 **Clinical picture of an acromegalic patient.**

somatostatin

- Hypothalamic small polypeptide
- Inhibit the secretion of GH
- Inhibit TSH

- It is present in gut and pancreas

↓

- it inhibit the secretion of gut hormones (e.g: gastrin, insulin, glucagon)
- It may be secreted from tumors of thyroid and lung

Prolactin (PRL)

- Polypeptide
- Initiate and maintain lactation
- Dopamine from hypothalamus control (inhibit) PRL secretion
- Increased PRL secretion may be from:
  - PRL-secreting tumors
  - tumors of pituitary which obstruct dopamine effect (in the absence of dopamine, PRL secretion is autonomous).
- Secretion of PRL is pulsatile:(↑in stress and sleep)
- Its secretion in women depend on estrogen levels
- At high conc., it inhibit the synthesis and release of GnRH from hypothalamus and thus gonadotrophins from pituitary, inhibiting ovulation in females and spermatogenesis in males.
- Its secretion increases during pregnancy

  ↓ after birth
- Concentration go back to normal if the woman does not breast feed

Prolactin (cont'd)

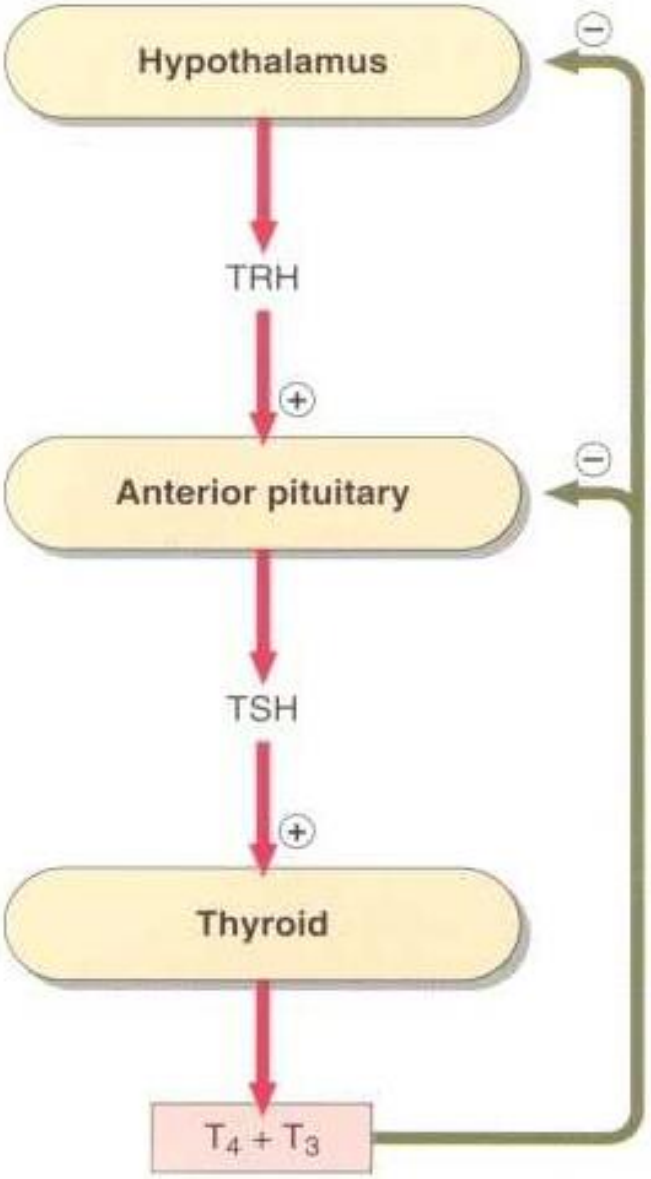
- Prolactinoma
  - A pituitary tumor that directly secretes prolactin
  - Most common type of functional pituitary tumor
  - Clinical presentation depends on patient age/gender, tumor size:
    - Premenopausal women: menstrual irregularity/amenorrhea, infertility, galactorrhea
    - Men/postmenopausal women: headaches or visual complaints
- Other Causes of Hyperprolactinemia
  - Pituitary stalk interruption, dopaminergic antagonist medications, thyroidal failure, renal failure, polycystic ovary syndrome

Prolactin (cont'd)

- Clinical Evaluation of Hyperprolactinemia
  - Careful history & physical examination
  - Obtain TSH & free T_4 levels.
  - If pituitary tumor is suspected, careful assessment of other anterior pituitary function & evaluation of sellar anatomy w/ MRI

Thyroid stimulating hormone (TSH)

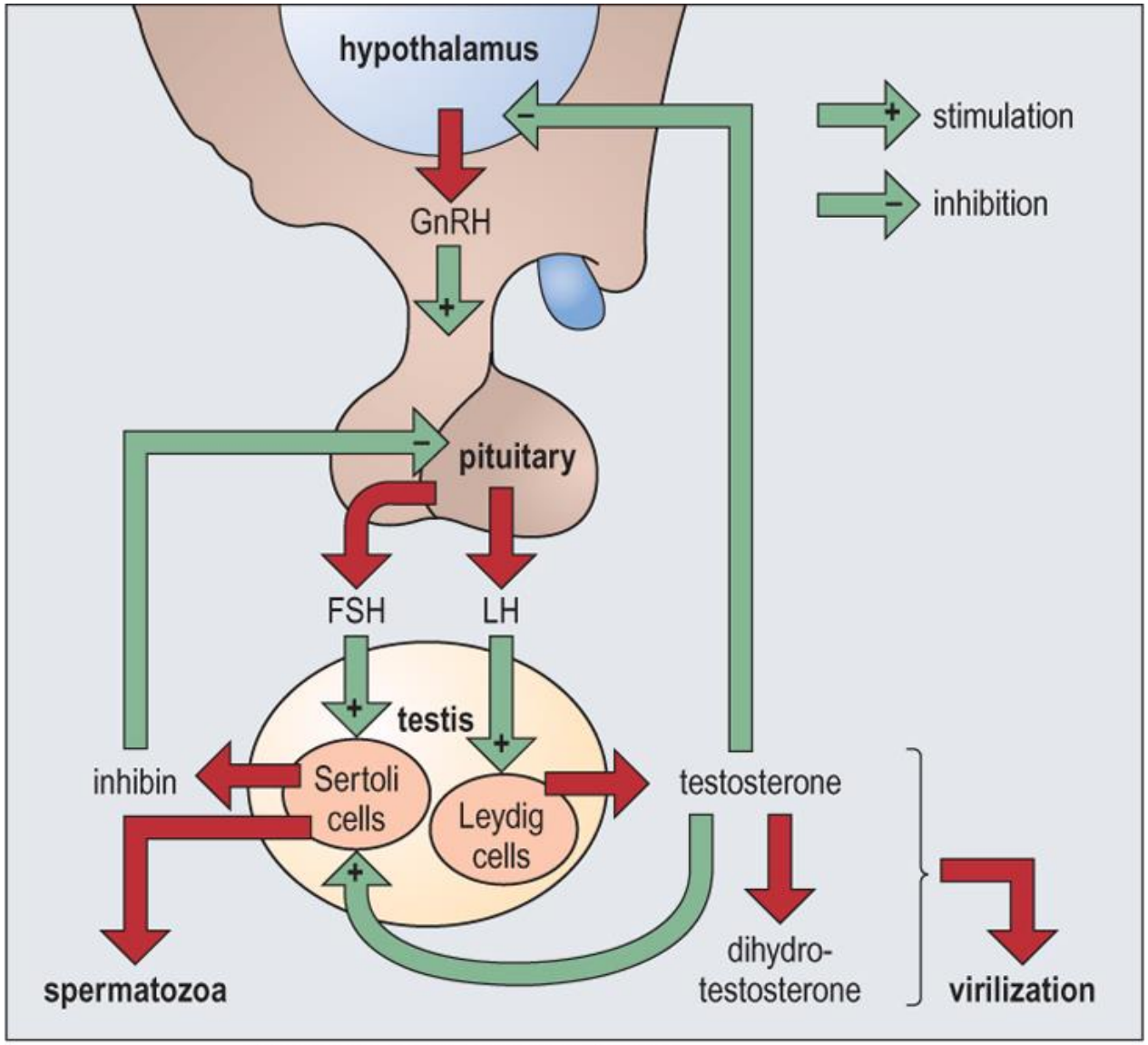
- It is a glycoprotein
- It exist in α and β subunits
- α-subunit is similar in TSH and gonadotropins, but the β-subunit is unique to TSH
- TSH stimulate the synthesis and secretion of thyroid hormones from thyroid gland
- TSH secretion is stimulated by hypothalamic TRH
- TSH is inhibited by circulating thyroid hormones by negative feedback mechanism
- In primary hypothyroidism,TSH secretion is increased
- In hyperthyroidism, TSH secretion is decreased



ig. 2 **Outline of the hypothalamic–ituitary–thyroid axis.**

Gonadotropins: FSH & LH

- Both are glycoproteins (α and β subunits)
- α-subunit is identical in both and to that of TSH
- Hypothalamic GnRH stimulate the synthesis and release of both FSH and LH from pituitary through the feedback effect of circulating steroids of the gonads
- Gonadotropins secretion is pulsatile .



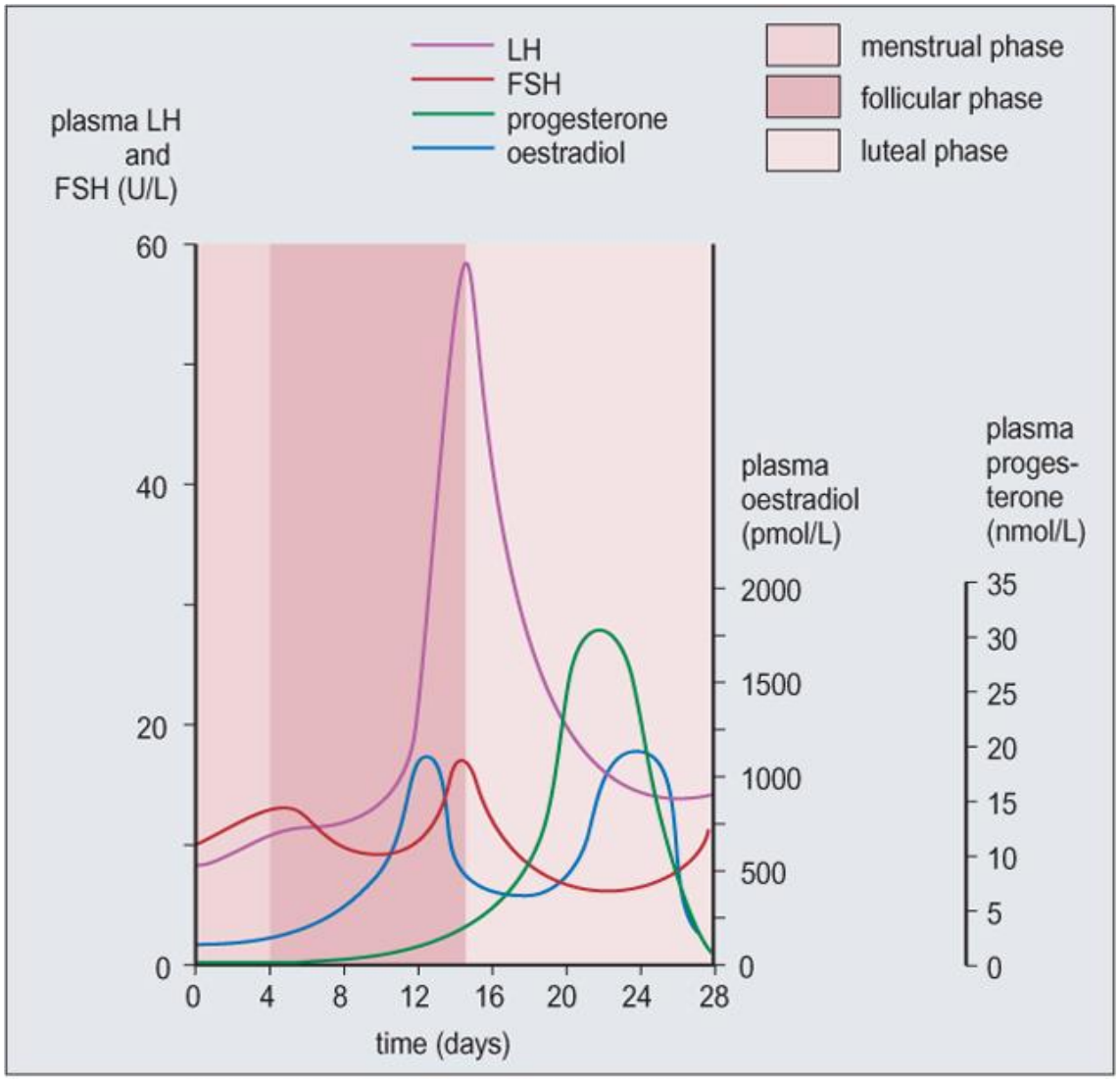
Gonadotropins (cont'd)

- In male: LH stimulate testosterone secretion from testis
- FSH+testosterone, stimulate spermatogenesis

- In female: FSH stimulate secretion of estrogen from ovary
- Both FSH and estrogen develop follicles

- High FSH is found in azospermia in men
- Increased gonadotropins are seen in ovarian failure (pathlogical cause or natural menopause)
- Decreased gonadotropins secretion, lead to secondary gonadal failure

Changes of plasma gonadotropins during menstrual cycle



Adrenocorticotropic hormone (ACTH)

- It is a poly peptide
- Its function is to stimulate secretion of glucocorticoids from adrenal gland
- pro-opiomelanocortin is the precursor of ACTH
- ACTH release is controled by hypothalamic CRH
- ACTH secretion is diurnal variation (highest at 8 a.m, lowest at midnight)
- Secretion increased by stress
- Secretion inhibited by cortisol (feedback control)

ACTH (cont'd)

- Increased ACTH secretion by pituitary is seen with pituitary tumors (Cushing's disease)
- Also increased in primary adrenal failure (addison's disease)
- The hormone may also be secreted ectopically by non-pituitary tumors.
- Elevated ACTH secretion is associated with pigmentation due to melanocyte-stimulating action of ACTH

Hypopituitarism

- Failure of either pituitary or hypothalamus results in loss of anterior pituitary function:
  - **Panhypopituitarism:** complete loss of function
  - **Monotropic hormone deficiency:** loss of only a single hormone
- Associated with low or normal levels of tropic hormone
- Both tropic & target hormone levels should be measured when there is any suspicion of pituitary failure.
- If one secondary deficiency is documented, search for other deficiency states & cause for pituitary failure.

other disorders of anterior pituitary function

- **Anorexia nervosa**: it resmble hypopituitarism
  - characterized by self-imposed starvation
  - decreased gonadotropins → amenorrhea
  - sever weight loss

- **Pituitary tumors**:
  - are usually functional → producing excessive quantities of hormones
  - biochemical measurements are important to assess pituitary function
  - the order of frequency for hormone secretion from tumors:
    prolactin (relatively common) > GH > ACTH > gonadotropins > TSH (very rare)

Disorders .. Continue…

- **Hyperprolactinemia**:

  -is a common endocrine abnormality

  -cause infertility both in males and females

  - male impotence

  -menstrual irregularity in females

  -these effects may be mediated through the inhibition of GnRH by prolactin

  -high levels of the hormone may be detected due to **macroprolactin** (a complex of prolactin with an immunoglobulin), but does not cause the above clinical features of hyperprolactinemia

- **Cushing's disease**:

  -increased secretion of ACTH $\rightarrow$ cause increased secretion of cortisol by adrenal cortex

  -hyperpigmentation $\rightarrow$ due to increased ACTH

Clinical features of excessive GH secretion

Clinical features of excessive growth hormone secretion

Somatic	Metabolic	Local effects of tumour
increased growth of: skin, subcutaneous tissues skull, jaw hands, feet long bones, if before fusion of epiphyses	elevated, non-suppressible plasma GH concentration	headache
nerve compression (particularly carpal tunnel syndrome)	glucose intolerance	visual field defects
excessive sweating, greasy skin, acne	clinical diabetes mellitus	hypopituitarism
goitre	hypercalcaemia hyperphosphataemia	diabetes insipidus
cardiomegaly, hypertension		
increased risk of colonic cancer		

Hyperprolactinemia

Hyperprolactinaemia

Causes

Physiological
- stress, sleep, pregnancy, suckling

Drugs
- dopaminergic receptor blockers, e.g. phenothiazines, haloperidol
- dopamine-depleting agents, e.g. methyldopa, reserpine
- others, e.g. oestrogens, TRH

Pituitary disorders
- prolactin-secreting tumour (prolactinoma)
- tumours blocking dopaminergic inhibition of prolactin secretion
- pituitary stalk section and surgery

Others
- hypothyroidism
- ectopic secretion
- chronic renal failure

Clinical features

Females
- oligomenorrhoea, amenorrhoea
- infertility
- galactorrhoea

Males
- impotence
- infertility
- gynaecomastia

Posterior pituitary hormones

1- Vasopressin(ADH)

2- oxytocin

Both are:

-synthesized: in hypothalamus

-secreted: from posterior pituitary to circulation

Posterior Pituitary Hormones

- Posterior pituitary is an extension of forebrain & represents storage region for oxytocin & vasopressin.
- **Oxytocin**
  - A cyclic nonapeptide, with a disulfide bridge connecting amino acid residues 1 & 6
  - Has a critical role in lactation
  - Likely plays a major role in labor & parturition
  - Synthetic oxytocin, Pitocin, is used in obstetrics to induce labor.
  - Has been shown to have effects on pituitary, renal, cardiac, & immune functions
  - Disorders: very uncommon

Posterior Pituitary Hormones (cont'd)

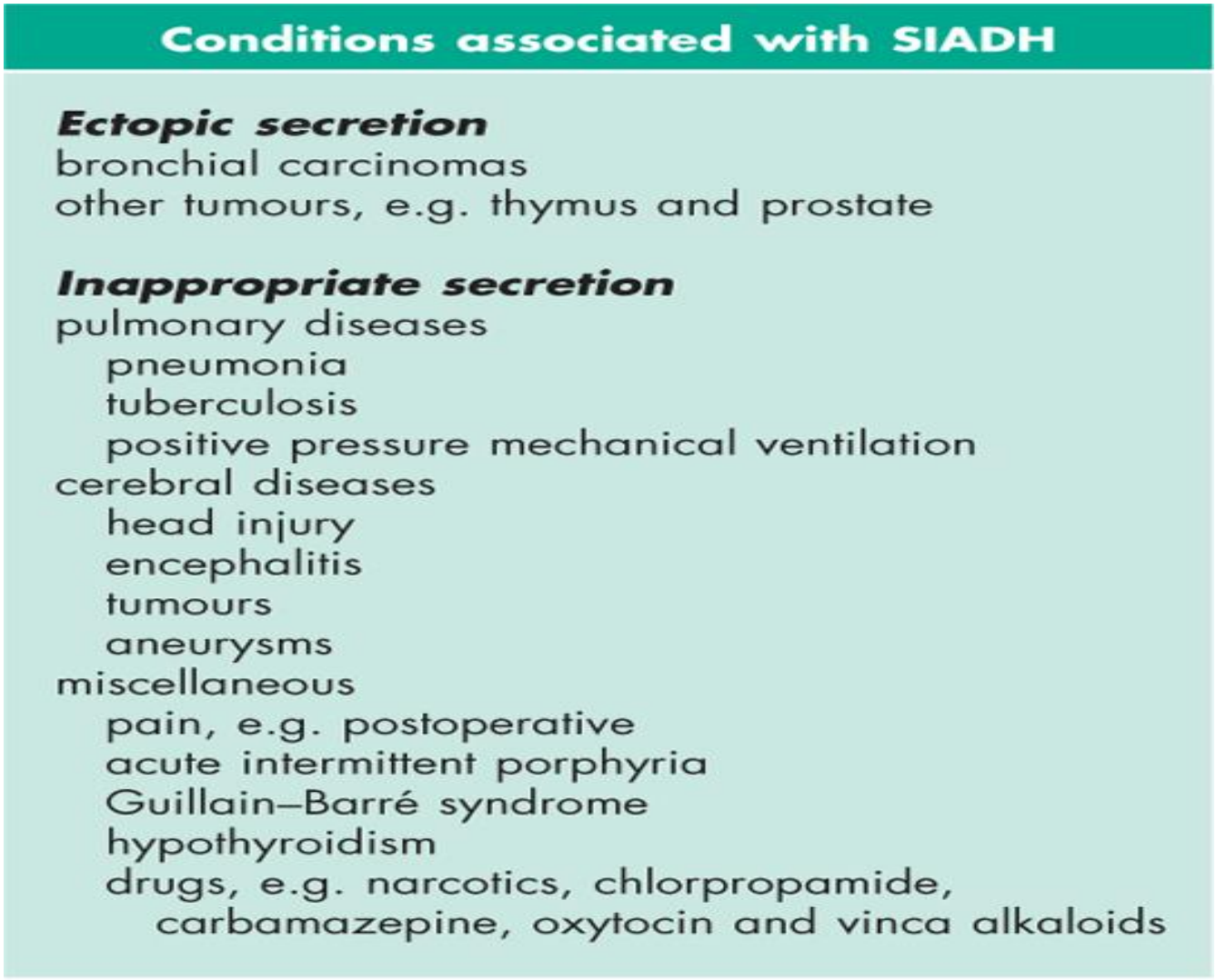
- **Vasopressin**
  - A cyclic nonapeptide, structurally similar to oxytocin, with an identical disulfide bridge; differs by only 2 amino acids
  - Major action is to regulate renal free water excretion & water balance.
  - direct control of tonicity of ECF, and indirectly of the ICF and water balance
  - A potent pressor agent & affects blood clotting
  - Deficiency can lead to diabetes insipidus, characterized by excessive urine production (polyuria) & intense thirst (polydipsia).

- Excessive secretion: results in dilutional hyponatremia

- this syndrome is frequently seen in conditions that do not affect the pituitary directly (SIADH)

- It can be diagnosed by measurement of urine and plasma osmolalities

Conditions associated with SIADH



Diabetes insipidus

- decreased secretion of ADH :

  -results in diabetes insipidus which is

  uncontrolled excretion of water with a tendency of sever dehydration
- usually due to pituitary or hypothalamic disease (CDI)

- or failure of the kidney to respond to ADH: nephrotic DI (NDI)

- In both conditions it results in:

  polyuria and **polydipsia**

  - while in primary polydipsia (psychogenic polydipsia): polyuria is a response or secondary to increased fluid intake

DI… cont…

- -if urine osmolality greater than 750 mmol/L, DI is excluded (most probably it is primary polydipsia)

- if there is doubt about diagnosis: fluid deprivation test should be performed

(in DI: the urine does not become concentrated and plasma osmolality rises)

- about 5% of cases of CDI are familial

- management of DI: with desmopressin

Causes of DI

Causes of diabetes insipidus

Cranial

tumours:
- craniopharyngioma
- secondary tumours
- pituitary tumours with suprasellar extension

granulomatous disease
meningitis and encephalitis
vascular disorders
trauma (may be transient)
surgery (often transient)
idiopathic
familial (~5% of cases)

Nephrogenic

familial
metabolic:
- hypokalaemia
- hypercalcaemia

drugs:
- lithium
- demeclocycline

post-obstructive uropathy
chronic renal disease:
- pyelonephritis
- polycystic disease
- amyloid
- sickle cell disease

Fluid deprivation test

Fluid deprivation test

allow fluids overnight before test and give light breakfast with no fluid; no smoking permitted

weigh patient

allow no fluid for 8 h; patient must be under constant supervision during this time

every 2 h:
- weigh patient (stop test if weight falls by >5% initial body weight[a])
- patient empties bladder: measure urine volume and osmolality
- measure plasma osmolality (stop test if osmolality >300 mmol/kg[a])

after 8 h allow patient to drink (no more than twice urine volume of period of fluid deprivation, to avoid acute hyponatraemia) and give 2 µg desmopressin i.m.

measure urine osmolality every 4 h for a further 16 h

interpretation of results: see Fig. 7.15

Disorders of Lipid Metabolism

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Major lipids

- Fatty acids (FA)
- Triglycerides
- Cholesterol
- Phospholipids
- Lipid-soluble substances (present in much smaller amounts but of physiological importance include: steroid hormones and fat-sol vitamins

- Usually lipids circulate in blood bound to proteins mostly albumin which is the principal carrier of FFA
- Other lipids circulate in complexes known as lipoproteins

Chemistry of Lipids

- Roles of Lipids (Fats)
  - Rich source of energy & efficient way for body to store calories
  - Integral part of cell membranes and structure
- Fatty Acids
  - Linear chains of carbon-hydrogen bonds terminating in carboxyl group
- Triglycerides
  - 3 fatty acid molecules attached to 1 molecule of glycerol by ester bonds

- Phospholipids
  - Similar to triglycerides, except with only 2 esterified fatty acids
  - Third position on glycerol backbone contains phospholipid head group.
  - Types of head groups: choline, inositol, serine, ethanolamine, all of which are hydrophilic in nature
- Cholesterol
  - An unsaturated steroid alcohol containing 4 rings & single side chain tail
  - Synthesized almost exclusively by animals; not readily catabolized by most cells, not a source of fuel

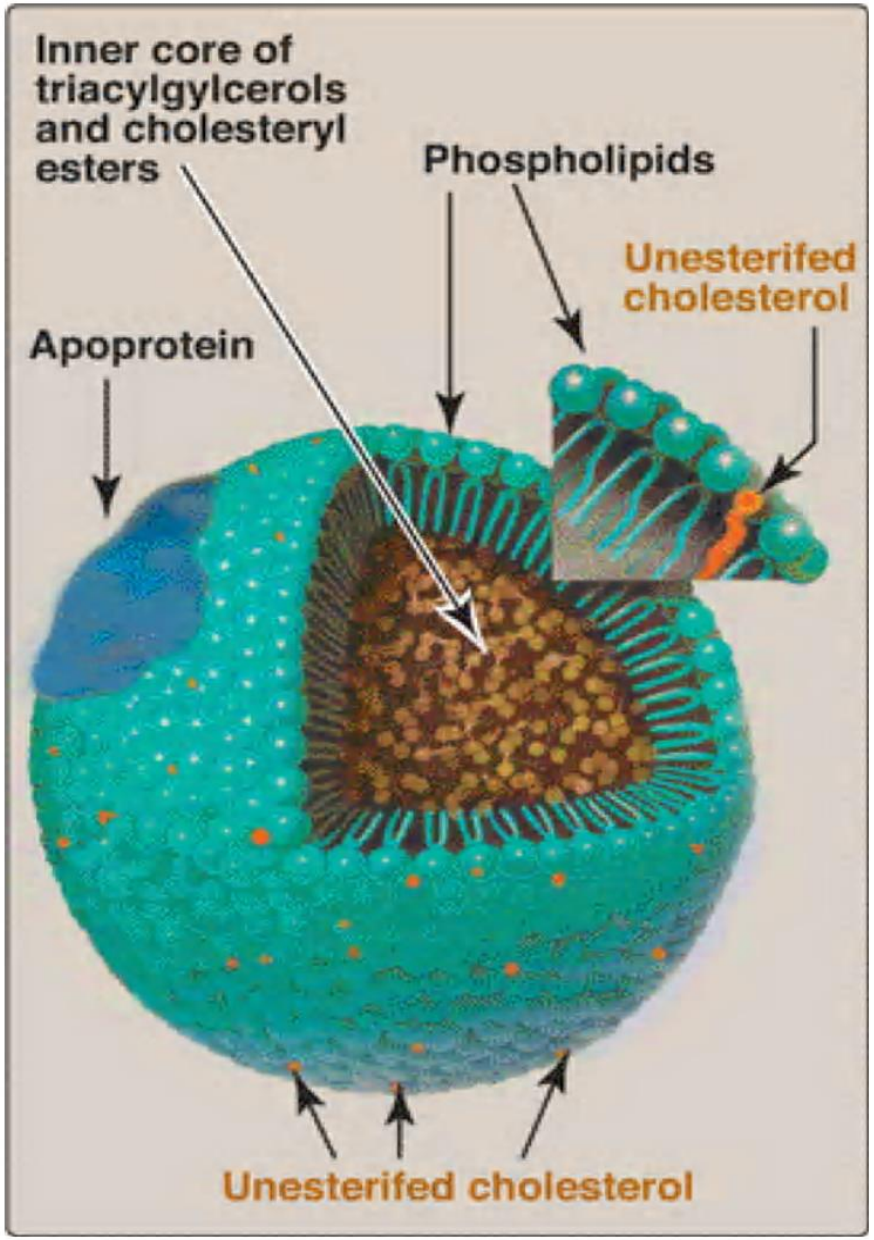


Figure 18.14 Structure of a typical lipoprotein particle.

Apolipoproteins

- the apo-protein associated with lipoprotein particles have a number of diverse functions:

-providing recognition sites for cell-surface receptors

-serving as activators or coenzymes for enzymes involved in lipoprotein metabolism

-some of the apo-proteins are required as part of the structural components of the particles and cannot be removed

- whereas others are transferred freely between lipoproteins

- they are divided into five major classes: A to E

(and subclasses: e.g A-1, C-II ..)

Apolipoprotein	Function
A-I	activates LCAT structural (in HDL)
A-II	inhibits HTGL at high concentration structural (in HDL)
B-100	structural (in LDL and VLDL) receptor binding
B-48	structural (in chylomicrons)
C-II	activator of LPL
C-III	inhibits LPL inhibits clearance of CM and VLDL remnant particles
E	binding to LDL and remnant receptors

- Elevated plasma conc. of lipids particularly cholesterol

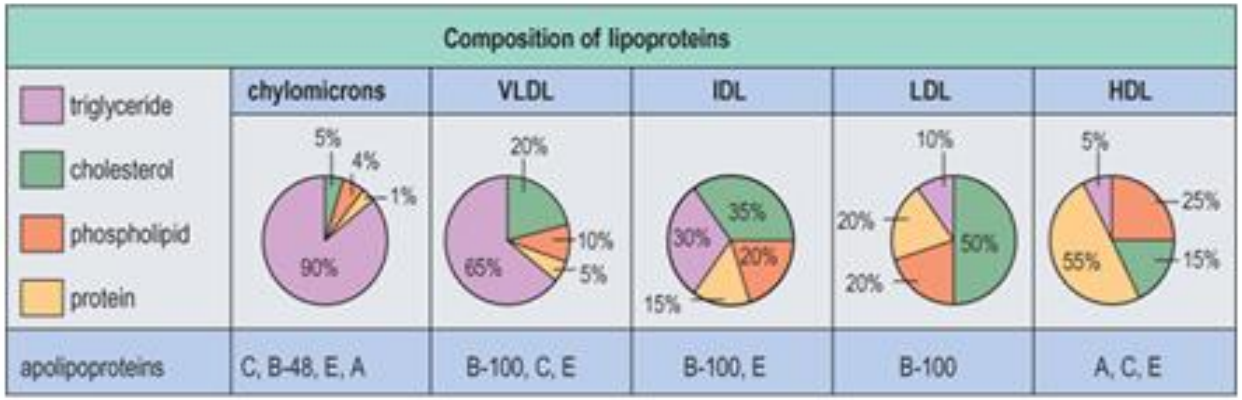
↓

pathogenesis process of **atherosclerosis**

↓

which is responsible for the majority of cardiovascular disease :

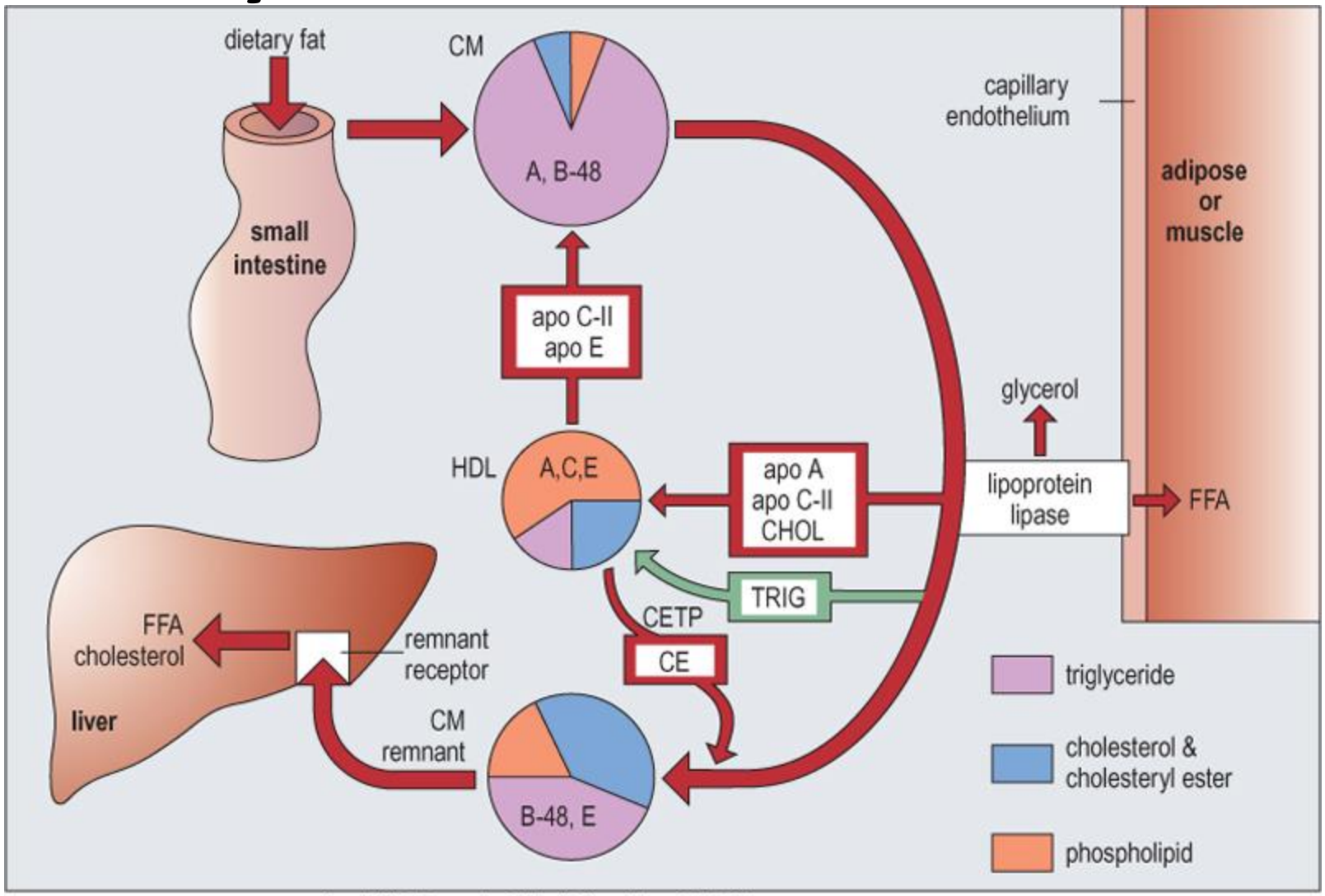
- coronary
- cerebrovascular
- peripheral vascular disease



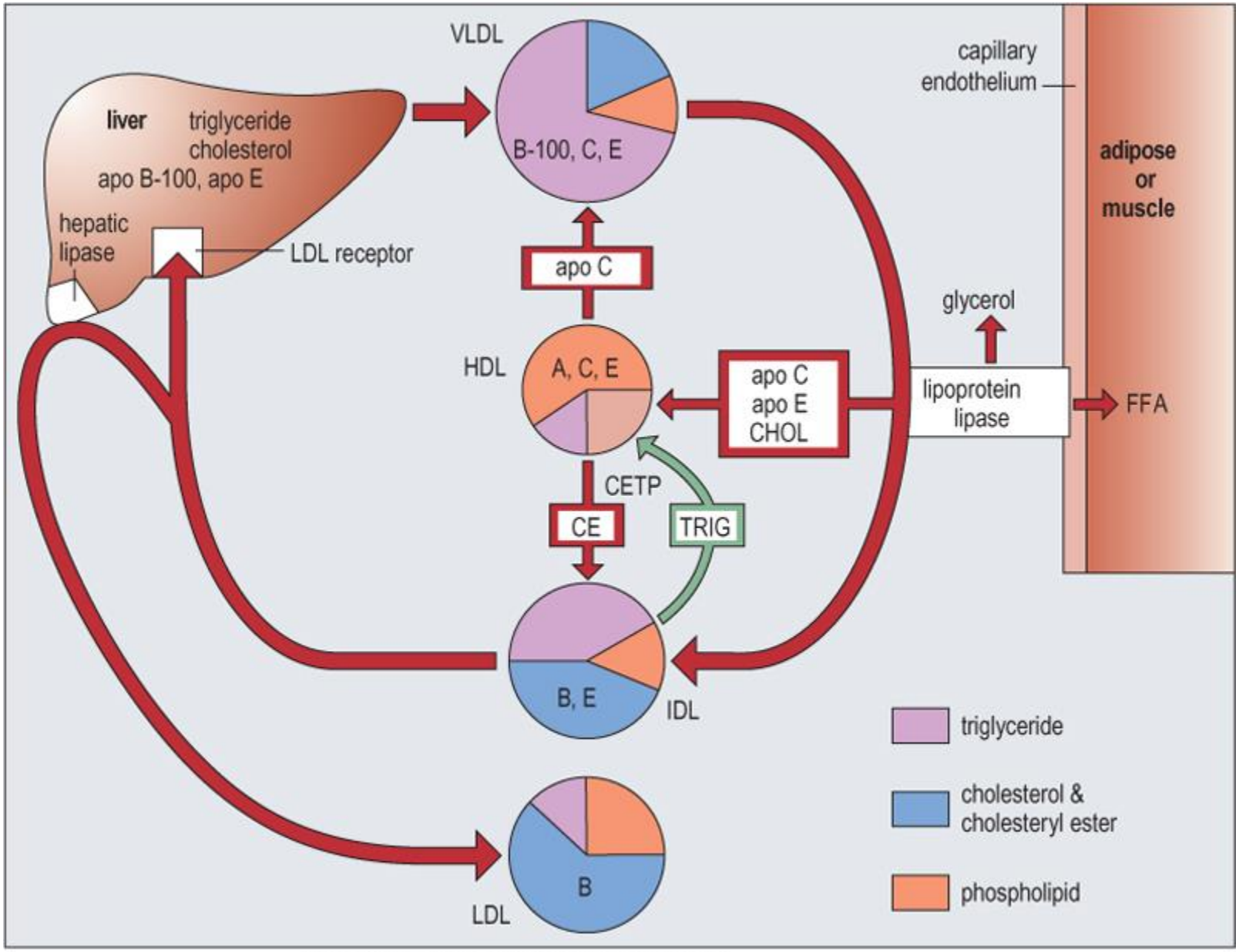
Classification and characteristics of lipoproteins

Lipoprotein	Density (g/mL)	Mean diameter (nm)	Electrophoretic mobility	Source	Principal function
CM	<0.95	500	remains at origin	intestine	transport of exogenous triglyceride
VLDL	0.96–1.006	43	pre-β	liver	transport of endogenous triglyceride
IDL	1.007–1.019	27	'broad β'	catabolism of VLDL	precursor of LDL
LDL	1.02–1.063	22	β	catabolism of VLDL, via IDL	cholesterol transport
HDL	1.064–1.21	8	α	liver, intestine; catabolism of CM and VLDL	reverse cholesterol transport

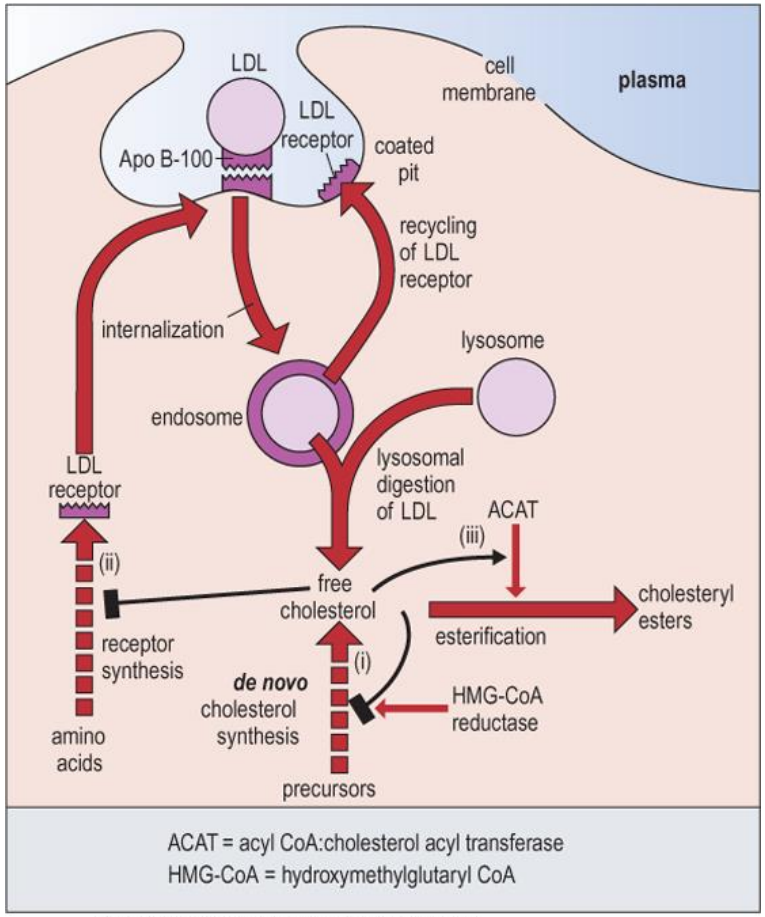
Chylomicrons metabolism



VLDL metabolism



LDL uptake and catabolism



Uptake of modified LDL by macrophages scavenger receptors

- macrophages posses high levels of scavenger receptor activity
- these receptors can bind and mediate the endocytosis of chemically modified LDL
- scavenger-receptors are unlike LDL-receptors, they are not down regulated by increased intracellular chol

↓

chol-esters accumulate in macrophages

↓

formation of **“ foam” cells**

↓

which participate in the formation of **atherosclerotic plaque**

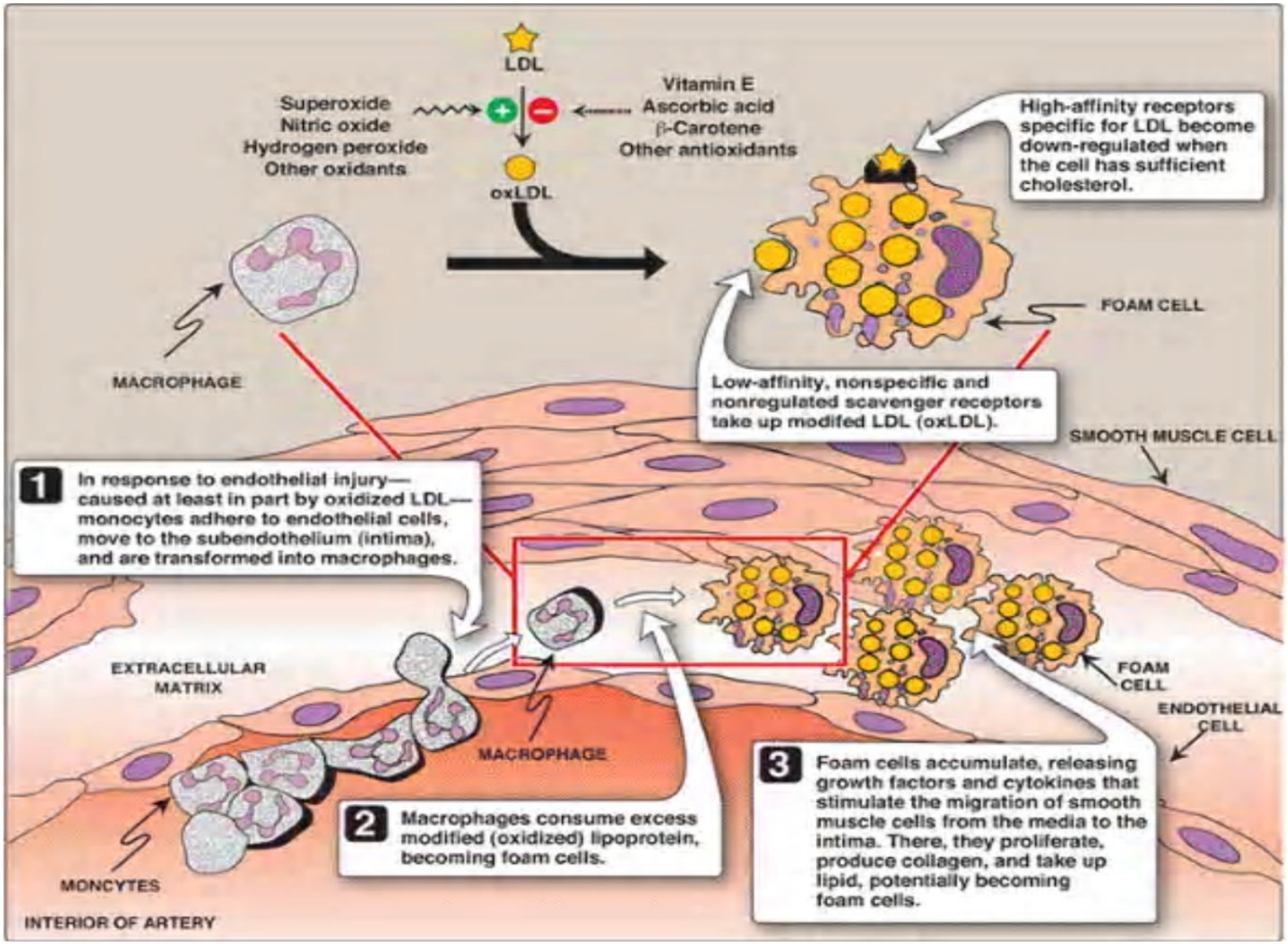
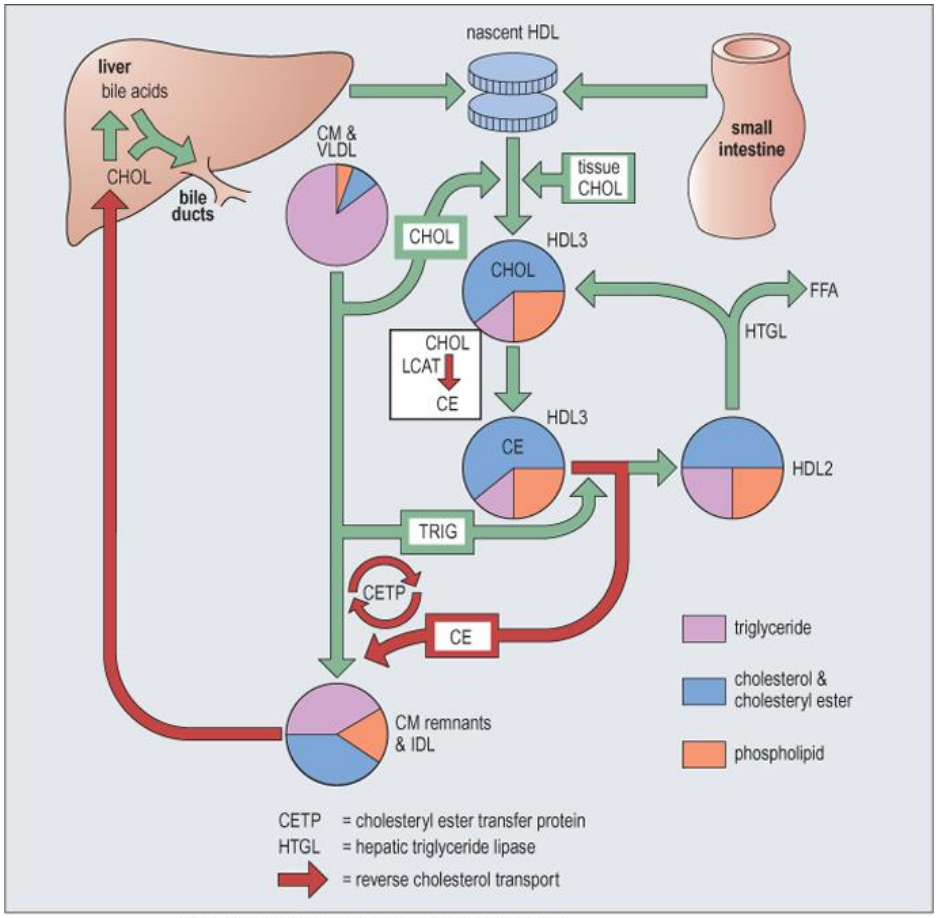


Figure 18.22 Role of oxidized lipoproteins in plaque formation in arterial wall.

HDL metabolism & reverse cholesterol transport



The essential features of lipoprotein metabolism

- **Dietary Tg**: are transported in CM to tissues where they can be used as energy source or stored

- **Endogenous Tg**: synthesized in the liver and transported in VLDL, also available as energy source or stored energy

- **Cholesterol**: synthesized in liver. Transported to tissues in LDL (derived from VLDL)
- *dietary cholesterol reach the liver by CM

- **HDL**: acquire cholesterol from peripheral cells and other lipoproteins which is esterified then transferred to remnant particles which are taken by the liver → excreted by bile

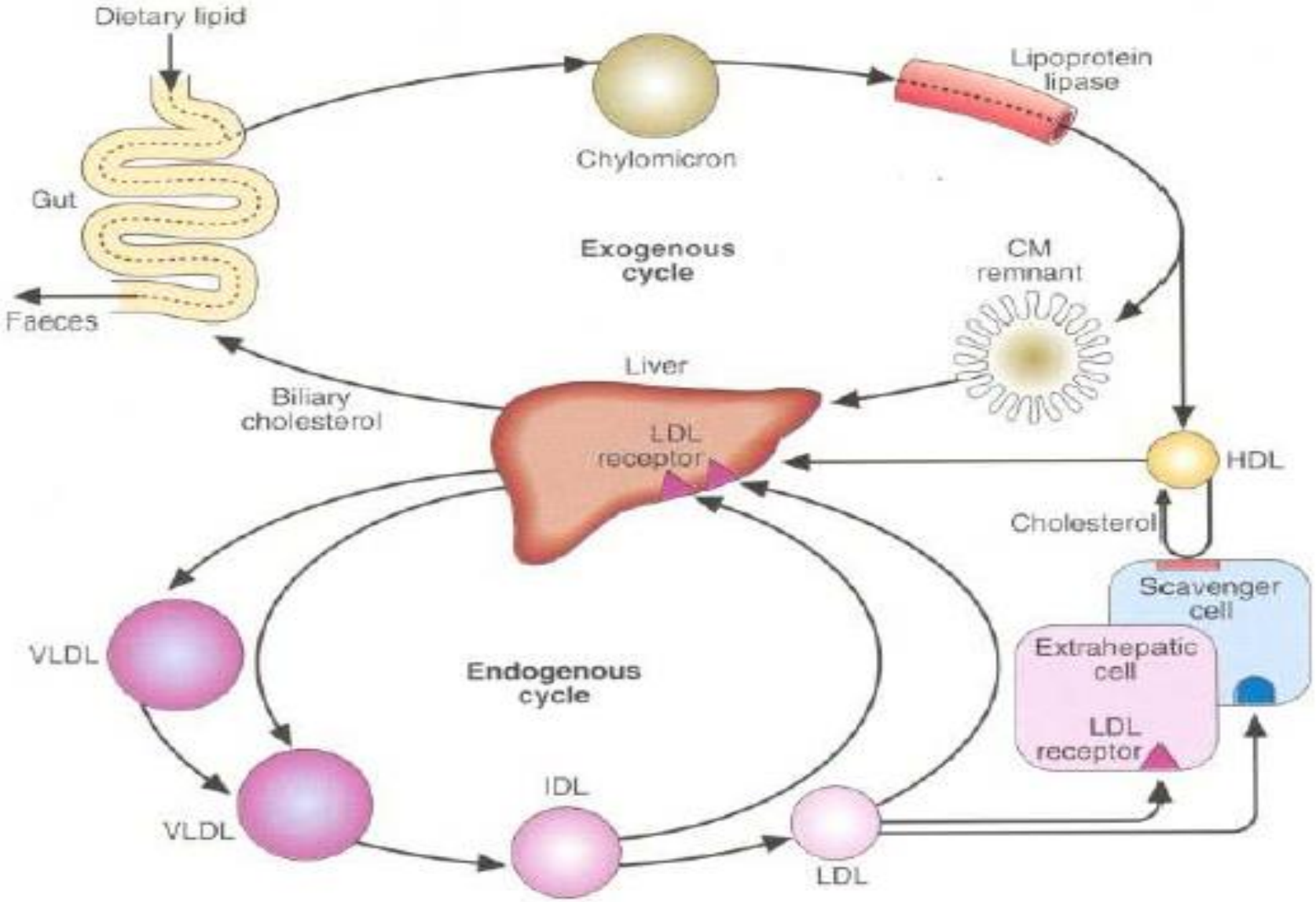


Fig. 2 **Lipoprotein metabolism.**

Lipoprotein (a) or Lp(a)

- is an atypical lipoprotein of unknown function
- it is larger and more dense than LDL but has similar composition (and a molecule of apo(a) in addition to apo B-100)
- an elevated conc. of Lp(a) is an independent risk factor for CHD
- conventional drug treatments that lower LDL, have little effect on Lp(a) conc.

Lipid and Lipoprotein Population Distributions

- Women have, on average, higher HDL cholesterol levels but lower total cholesterol & triglyceride levels than men.
- After menopause, no difference in total cholesterol
- Total & LDL cholesterol & triglyceride levels all increase with age, in both men & women.
- Total & LDL cholesterol & triglycerides are much lower in young children than adults.
- At puberty, boys' HDL cholesterol drops 20% to adult male levels, but girls' does not change.
- Lower rates of LDL cholesterol & heart disease in Asians

Lab investigations

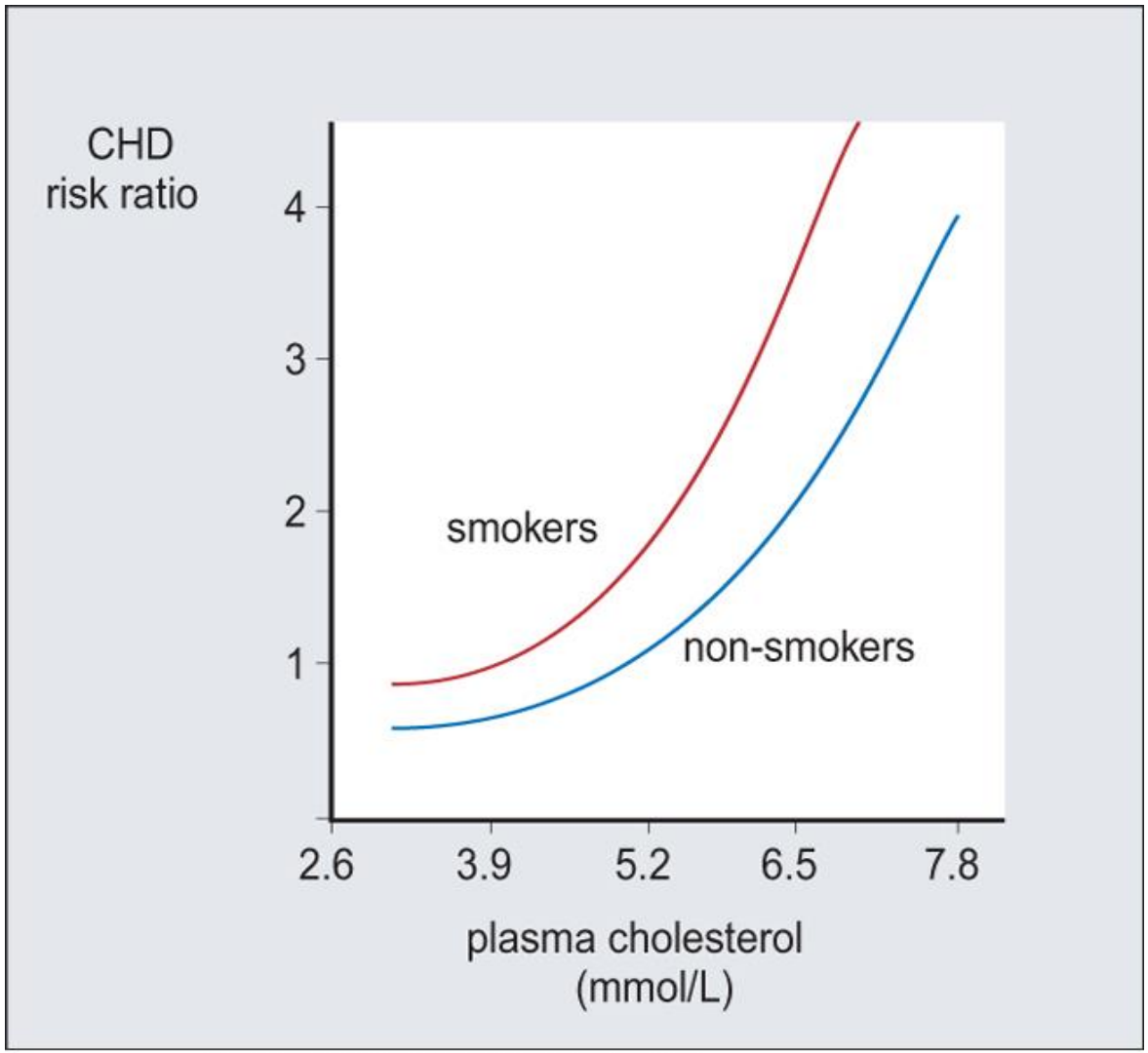
- plasma chol reference range is preferably considered for the individual: which may depend on many factors including the presence or absence of other CHD risk factors

- there is an inverse correlation between HDL-ch and CHD risk

- hypertriglyceridemia is also a risk factor for CHD

- plasma Tg conc. > 10 mmol/L (>800mg/dL) carry an increasing risk of pancreatitis

Mortality from CHD and plasma chol concentrations



Some influences on plasma lipoproteins			
Variable	**HDL cholesterol**	**LDL cholesterol**	**Triglyceride**
sex	F > M	M = F	F < M
age	slight ↑ in F	↑	↑
high P:S ratio	N or ↓	↓	N or ↓
exercise	↑	↓	↓
obesity	↓	N	↑
alcohol	↑	N	↑
exogenous oestrogens	↑	↓	↑°

Marshall & Bangert: Clinical Chemistry, 6th Edition.

Lab investigations.. Cont..

- Tg , total-ch , and HDL-ch conc., can easily be measured in the laboratory
- LDL-ch can be calculated

- the appearance of the blood sample in the lab, provide the first clue that a patient has hyperlipidemia

-with sever hypertriglyceridemia the sample appears milky (lipemic)

- under normal conditions, chylomicrons cannot be detected in plasma in the fasting state (> 12 h fast)

Adult reference ranges for lipids

- total-ch .140-200 mg/dL (3.6 - 5.2 mmol/L)
- HDL-ch …40-75 mg/dL (1.0 - 1.9 mmol/L)
- LDL-ch …50-130 mg/dL (1.3 - 3.4 mmol/L)
- Tg …… 60-150 mg/dL (0.4 - 2.5 mmol/L)

Blood for lipid investigation

- after overnight fast
- patient on normal diet for two weeks before taking blood sample
- no alcohol taken on the night before blood sampling
- in patients with MI or stroke, blood should be taken either within 24h or after three months

Selection of patients for investigation

- lipid measurement should be made in all patients known to have vascular disease, and in those at high risk. Thus plasma lipid should be measured in the following individuals:

- CHD, cerebrovascular and peripheral-vascular disease

- a family history of premature coronary disease

- other major risk factors for CHD (e.g DM, hypertension)

- patients with clinical features of hyperlipidemia

- patients whose blood is seen to be lipemic

Disorders of lipid metabolism

- diseases associated with lipid abnormal conc., are referred to as **dyslipidemia** caused by:

  *genetic abnormalities (primary)

  *environmental / lifestyle imbalances

  *or develop secondarily to other diseases

- many dyslipidemias are associated with CHD or arteriosclerosis

Common causes of secondary hyperlipidemia

Condition	Lipid abnormality		
	HDL cholesterol	LDL cholesterol	Triglyceride
obesity	↓	N	↑
excessive alcohol intake	↑	N	↑
diabetes mellitus	N/sl ↓	N	↑↑
hypothyroidism	N	↑↑	N/↑
nephrotic syndrome	↓	↑↑	↑↑
chronic renal failure	↓	N/↑	↑
cholestasis	N	↑	N

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Disorders.. Cont..

- Dyslipidemias
  - hyperlipoproteinemias
  - hypolipoproteinemias

Hyperlipoproteinemias
- hypercholesterolemia
- hypertriglyceridemia
- combined hyperlipidemia

Disorders of lipid metabolism (Dyslipidemias)

- Arteriosclerosis
  - Single leading cause of death & disability in U.S.
  - Caused by lipids, in form of esterified cholesterol, being deposited in artery walls, resulting in fatty streaks
  - Fatty streaks develop into plaques that can block blood flow.
- **Hyperlipoproteinemia**
  - Diseases associated with elevated lipoprotein levels
  - Includes hypercholesterolemia, hypertriglyceridemia, & combined hyperlipidemia
- Lp(a) Elevation
  - Increased risk of CHD & cerebrovascular disease

Disorders (Dyslipidemias) (cont'd)

- Hypercholesterolemia
  - Lipid abnormality most closely linked to heart disease
  - Familial hypercholesterolemia (FH): genetic abnormality predisposing people to elevated cholesterol levels
    - Homozygotes: rare (1:1 million); first heart attack in teens
    - Heterozygotes: more common (1:500)

- patients lack of LDL receptors

- so LDL-chol (synthesized+absorbed) will build up in circulation

Remnant hyperlipoproteinemia

- it is a familial dyslipidemia

- characterized by the presence of an excess of:
  - **IDL and chylomicron remnants**

- patients have an increased risk of
  *CHD
  *Peripheral-vascular
  *and cerebral vascular

Familial chylomicronemia

- it is a rare hyperlipidemia

- it is due to either:

  *lipoprotein lipase enzyme deficiency

  *or a deficiency of apo C-II (required for the activation of this enzyme)

  ↓

  the result in each case is a failure of chylomicron clearance from bloodstream

Hypertriglyceridemia

- it is a result of an imbalance between synthesis and clearance of VLDL in the circulation

- it is either:

  *familial hypertriglyceridemia (genetic)

 - Elevated triglyceride levels: high, 200–500 mg/dL; very high, >500 mg/dL

  *or from secondary cause such as:

    --hormonal abnormalities:

    (pancreas, adrenal gland, pituitary gland)

    --or DM

    --or nephrosis

Combined hyperlipidemia

- It is due to overproduction of VLDL & LDL

- usually familial: due to elevated levels of:
  --serum total-chol
  --and Tg

- patients at high risk of CHD

Familial hyperalphalipoproteinemia

- it is hypercholesterolemia due to an increase in only HDL fraction
- CHD risk decreased
- and no treatment is required

Disorders (Dyslipidemias) (cont'd)

- Hypolipoproteinemia
  - Low levels of lipoproteins
  - Two forms: hypo*alpha*lipoproteinemia & hypo*beta*lipoproteinemia
- Hypoalphalipoproteinemia
  - Isolated decrease in circulating HDL (concentration <40 mg/dL), without presence of hypertriglyceridemia
  - *Alpha* denotes region in which HDL migrates on agarose electrophoresis.
  - Associated with several defects, often genetic, most of which are linked to increased risk of premature CHD

Non-HDL Cholesterol

- Reflects total cholesterol minus HDL-C
  - LDL, VLDL, IDL, Lp(a)
  - Elevated non-HDL-C associated with increased risk of CVD.

Risk factors for cardiovascular disease

Modifiable	Not modifiable
hypercholesterolaemia[a]	personal history of cardiovascular disease[a]
hypertension[a]	family history of premature cardiovascular disease[a]
cigarette smoking[a]	male sex[a]
diabetes mellitus[a]	age[a]
hyperfibrinogenaemia	
hyperhomocysteinaemia	
low HDL cholesterol	
hypertriglyceridaemia	
overweight	
sedentary lifestyle etc.	

Myocardial infarction

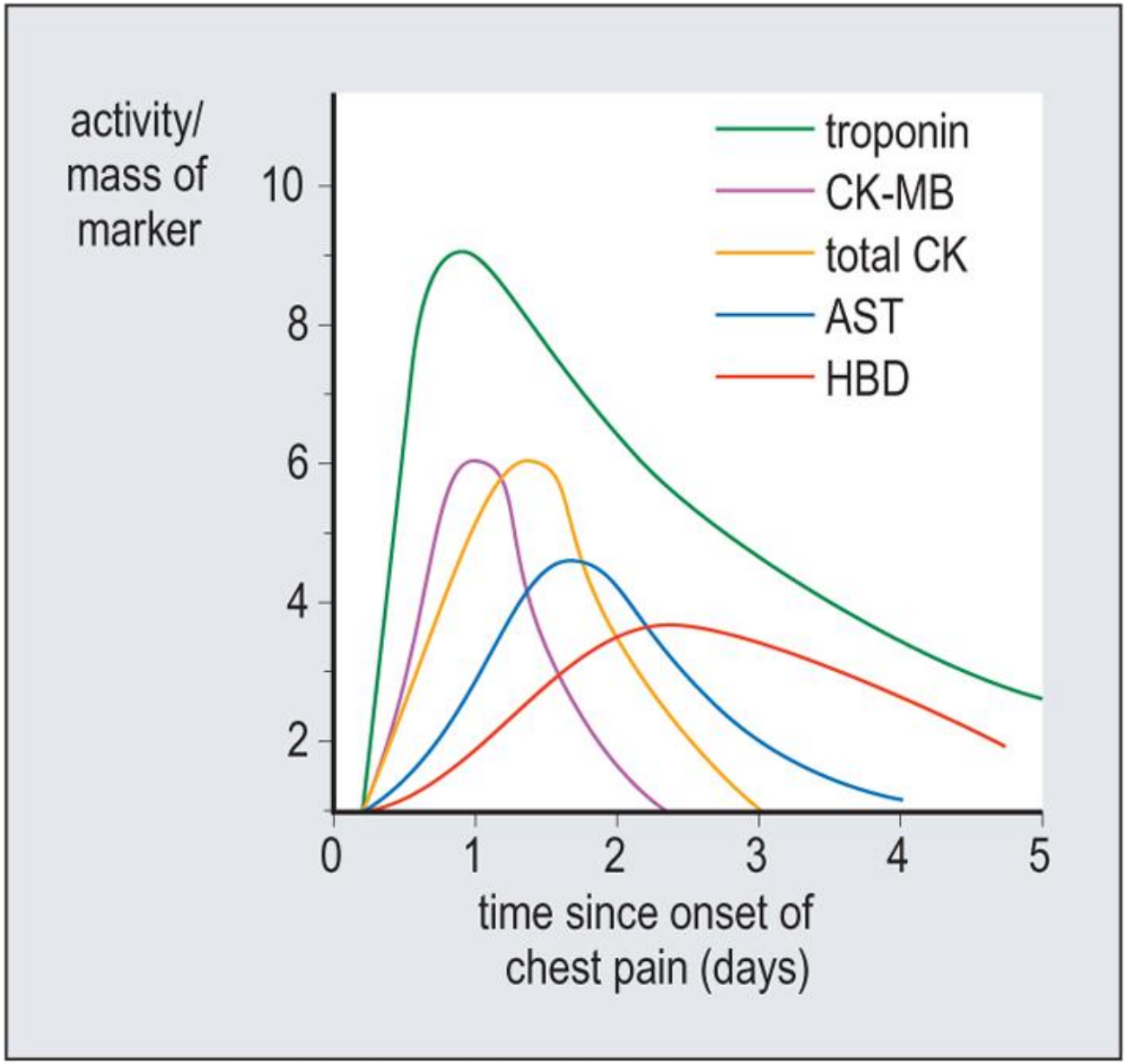
- measurement of plasma enzymes been used to assist in the diagnosis of MI

- CK-MB isoenzyme of CK is the first to increase (the gold standard)

- followed by total CK

- Troponin is highly assisting in MI diagnosis
- Troponins are highly specific cardiac- marker

- measurement of plasma: glucose, potassium and blood gases may be vital for treatment



Heart failure

- Biochemical tests assist greatly in diagnosis of heart failure

- the heart secretes two peptides → ANP, BNP

- Both are increased in heart failure
- Both measured in plasma

- a normal value → exclude heart failure

- other lab investigations may be necessary:

  plasma: albumin, creatinine and potassium

Laboratory investigations in patients with heart failure

Investigation	Explanation
full blood count	anaemia is a cause of 'high output' heart failure
plasma albumin	hypoalbuminaemia is another cause of oedema
plasma creatinine	renal impairment
plasma potassium	diuretic-induced hypokalaemia
thyroid function tests[a]	thyrotoxicosis
plasma ferritin[a]	haemochromatosis
serum and urine electrophoresis[a]	myeloma-related amyloid
endocrine causes of hypertension[a]	see Figure 14.16

Hypertension

- biochemical investigations: play no part in diagnosis of essential hypertension **but**:

  *for adverse effects of medication

  *and for complications of hypertension

- biochemical investigations are of value in diagnosis of secondary hypertension e.g:

  --renal disease

  --endocrine disease:

  (Conn's syndrome, Cushing's syndrome, pheochromocytoma, hyperparathyroidism)

  --pregnancy-associated hypertension

Biochemical investigations in patients with hypertension

Investigation	Explanation
urine analysis	for protein (renal disease) and glucose (diabetes, a cardiovascular risk factor)
plasma creatinine	renal disease
plasma potassium	mineralocorticoid excess (primary or secondary)
plasma calcium	hyperparathyroidism
plasma cholesterol and triglycerides	cardiovascular risk assessment
plasma renin and aldosterone[a]	Conn's syndrome
overnight dexamethasone suppression test[a]	Cushing's syndrome
urinary catecholamines/ metabolites[a]	phaeochromocytoma

Disorders of carbohydrate metabolism

Hyperglycemia

Hypoglycemia

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- Glycolytic pathway occur in all tissues for ⟶ breakdown of glucose to provide

  ●energy(in the form of ATP) ●intermediates for other metabolic pathways

- During metabolism of carbohydrate, all sugars(dietary or catabolic), can ultimately be converted to **glucose**
- **pyruvate** is the end product of glycolytic pathway in cells having mitochondria and good supply of oxygen, in this case it is called:

- **aerobic glycolysis** → set the stage to produce **acetyl Co A** → fuel for **TCA cycle**

- in **anaerobic glycolysis:** pyruvate is reduced to → **lactate** (the end product)
- Cells that lack mitochondria(e.g. RBCs),and in cells deprived of sufficient oxygen, still can produce ATP by anaerobic glycolysis

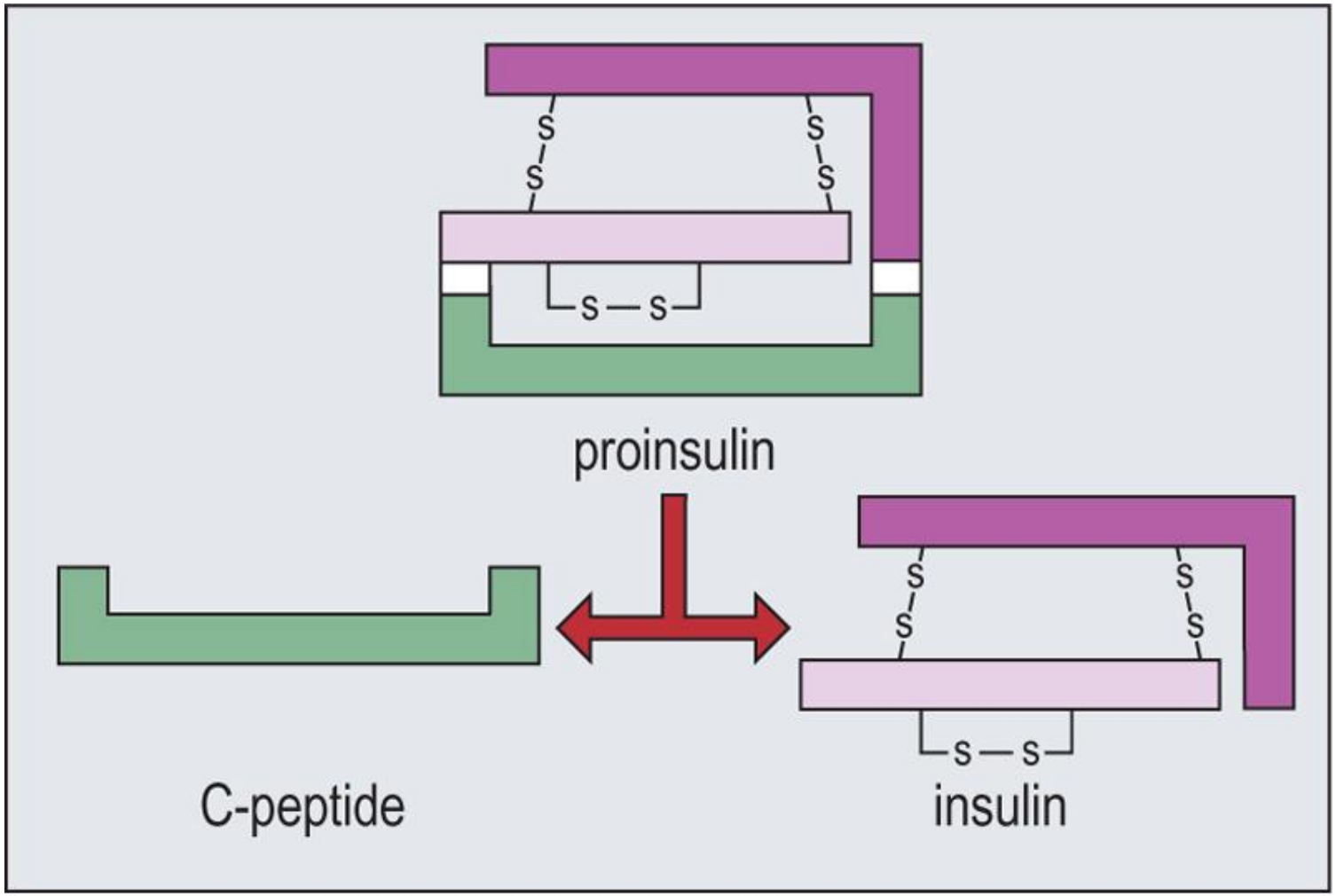
Hormones involved in glucose homeostasis

Hormones involved in glucose homoeostasis			
Hormone	**Principal actions**		
insulin	increases	cellular glucose uptake	M, A
		glycogen synthesis	L, M
		protein synthesis	*L, M*
		fatty acid and triglyceride synthesis	*L, A*
	decreases	gluconeogenesis	L
		glycogenolysis	L, M
		ketogenesis	*L*
		lipolysis	*A*
		proteolysis	*M*
glucagon	increases	glycogenolysis	L
		gluconeogenesis	L
		ketogenesis	*L*
		lipolysis	*A*
adrenaline (epinephrine)	increases	glycogenolysis	L, M
		lipolysis	*A*
growth hormone	increases	glycogenolysis	L
		lipolysis	*A*
cortisol	increases	gluconeogenesis	L
		glycogen synthesis	L
		proteolysis	*M*
	decreases	tissue glucose utilization	L, M, A

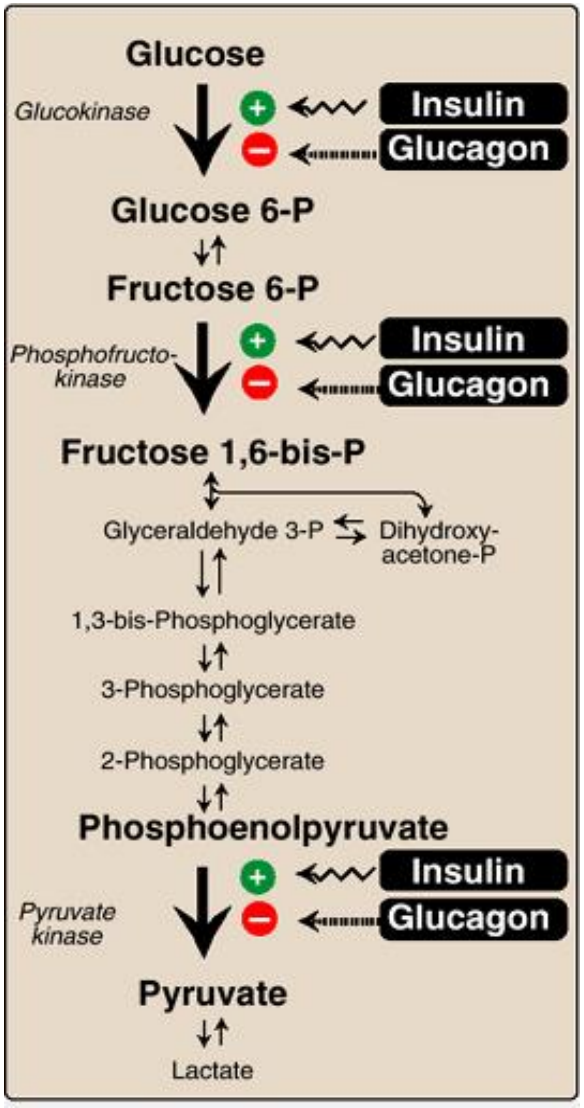
Insulin secretion

- B-cells synthesize insulin of the Langerhans cells.
- Secreted as pro-insulin from Golgi apparatus then cleaved to insulin & C peptide
- Activation of glucose transport , induction of hexokinase ,p-fructokinase , pyruvate kinase & pyruvate dehydrogenase .
- Glycogen synthase.

Biosynthesis of insulin

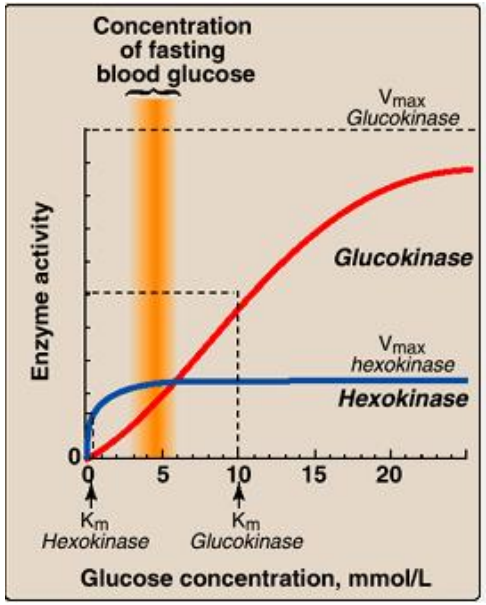


Hormonal regulation of glycolysis



Effect of insulin and glucagon on the synthesis of key enzymes of glycolysis in liver

- **Hormonal influence on the amount of enzyme protein synthesized.**
- **These effects can result in 10-20 folds increase in enzyme activity which occur over hours to days (long-term regulation).**
- **In the well-fed status, the hormone insulin favors the conversion of glucose to pyruvate .**
- **In fasting or in diabetes, those regulating enzymes are genetically decreased, the hormone glucagon is increased and insulin is low.**



Effect of glucose concentration on the rate of phosphorylation catalyzed by hexokinase and glucokinase

Hexokinase:

- in most tissues
- broad substrate specificity
- inhibited by the reaction product
- has a **low K_m** (and thus, a **high affinity** for glucose)

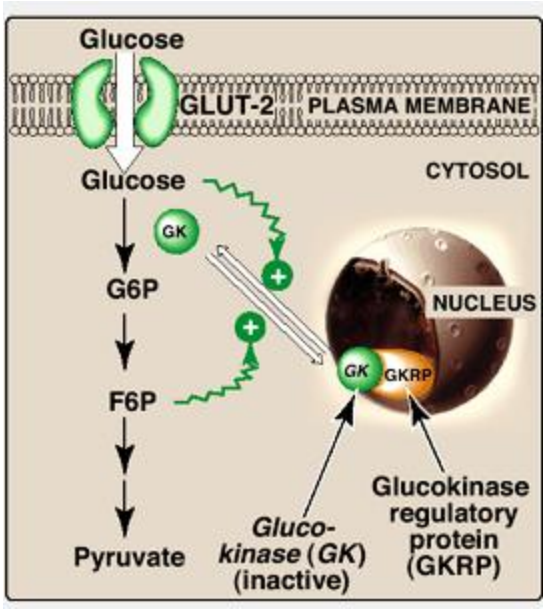
↓

permits for efficient phosphorylation and metabolism of glucose even at low tissue concentration of glucose

- has **low V_{max}** for glucose, so cannot phosphorylate more glucose than the cell can use

Glucokinase:

- in liver cells and in cells of the pancreas
- it function as the glucose sensor determining the threshold for insulin secretion
- in liver, it facilitate glucose phosphorylation during hyperglycemia
- it has **much higher K_m** (than hexokinase), requiring higher glucose concentration, thus it function only when the intracellular cnoc. of glucose in liver is elevated
- it has **high V_{max}**, removing the flood of glucose, thus minimizing hyperglycemia during absorptive period



Regulation of glucokinase activity by glucokinase regulatory protein

Regulation of Glucokinase:

(a) Regulation by fructose 6-ph and glucose:

- fructose 6-ph indirectly inhibit → glucokinase:

In hepatocytes, in the presence of fructose 6-ph, glucokinase is translocated into the nucleus and

↓

binds to regulatory protein ⟹ rendering it **inactive**

- when blood glucose level increase, the glucose causes the release of glucokinase from the regulatory protein ⟹ rendering it **active** enzyme

(b) Regulation by insulin:

when blood glucose level rise following a meal

↓

stimulate β-cells of the pancreas to release insulin

↓

it promotes transcription of the glucokinase gene

↓

increased liver enzyme protein, and therefore:

↓

increased total glucokinase activity

Diabetes mellitus

- Defect in insulin:
  - secretion
  - action
  - or both
- DM is either:
  - primary Dm (most common)
  - secondary DM (uncommon)
- Type 1 ;insulin – dependent (IDDM). ***NO Insulin secretion.***
- Type 2 ; (NIDDM); Non insulin dependent. ***Insulin secretion inadequate .***

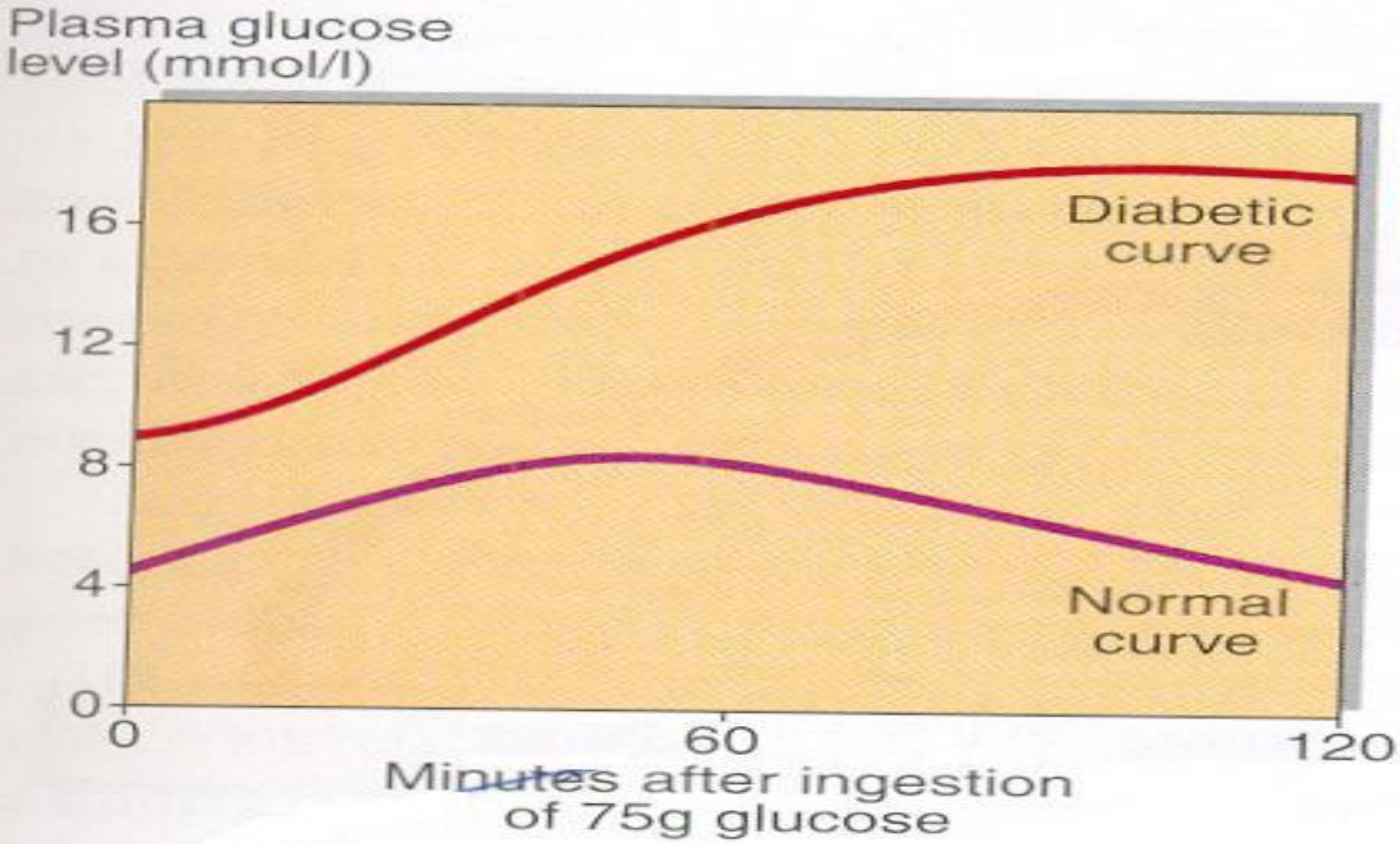


Fig. 2 **Plasma glucose levels following an oral glucose load in normal and diabetic subjects.**

Hyperglycemia

- Definition: an increase in plasma glucose levels caused by imbalance of hormones
- Diabetes Mellitus
  - A group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both
  - Type 1
    - Results from cellular-mediated autoimmune destruction of β cells of pancreas, causing *absolute* deficiency of insulin
    - Constitutes 10–20% of all diabetes cases; occurs in childhood & adolescence and is genetic

- Diabetes Mellitus
  - Type 2
    - Characterized by hyperglycemia caused by an individual's resistance to insulin, resulting in a *relative* insulin deficiency
    - Constitutes majority of diabetes cases & is adult onset
    - Risk factors include age, obesity, lack of exercise, genetic predisposition.
  - Gestational: glucose intolerance with onset during pregnancy

- Pathophysiology of Diabetes Mellitus
  - Hyperglycemia, possibly severe
  - Glucosuria can occur after renal tubular transporter system for glucose becomes saturated.
  - Type 1
    - Absence of insulin with excess of glucagon
    - Greater tendency to produce ketones
  - Type 2
    - Presence of insulin & hyperinsulinemia, attenuated glucagon
    - Greater tendency to develop hyperosmolar nonketotic states

- Criteria for Testing for Pre-Diabetes and Diabetes
  - All adults >45 years old should have fasting blood glucose measured every 3 years, unless already diagnosed with diabetes.
  - Testing should be earlier or more frequent w/ these risk factors:
    - Overweight tendencies (BMI ≥25 kg/m^2)
    - Habitual physical inactivity
    - Family history of diabetes in a first-degree relative
    - High-risk minority population (African American, Latino)
    - History of gestational diabetes or delivering baby > 4.5 Kg
    - Hypertension (≥140/90)

- Criteria for the Diagnosis of Diabetes Mellitus
  - Four methods of diagnosis (each must be confirmed by one of the first 3 methods on a subsequent day)
    - 1. HbA1c ≥6.5% using certified method
    - 2. Diabetes symptoms + random glucose level of ≥200 mg/dL
    - 3. A fasting plasma glucose of ≥126 mg/dL
    - 4. An oral glucose tolerance test (OGTT) w/ 2-hour postload (75-g glucose level) ≥200 mg/dL
  - Patients with following criteria have “pre-diabetes”:
    - Fasting glucose of ≥100 mg/dL but <126 mg/dL
    - OGTT 2-hour level of ≥140 mg/dL but <200 mg/dL

- Criteria for Testing & Diagnosis of Gestational Diabetes

  - New recommendation by the International Association of the Diabetes and Pregnancy Study Group:
    - All non-diabetic pregnant women be screened for GDM at 24 to 28 weeks of gestation period.

GDM

- Gestational diabetes : (GDM)
- Recognized during pregnancy
- Pregnancy reduces glucose tolerance
- Patients may revert to normal glucose tolerance post-partum

Role of Laboratory in Differential Diagnosis and Management of Patients

- Methods of Glucose Measurement
  - Fasting blood glucose should be obtained in morning after 8- to 10-hour fast.
  - Most common methods of glucose analysis use enzymes glucose oxidase or hexokinase methode.
  - Glucose can be measured from serum, plasma, or whole blood.
  - Nonspecific methods are used in urinalysis section of lab to detect reducing substances other than glucose.
  - Serum or plasma must be refrigerated & separated from cells within 1 hour to prevent loss of glucose.
  - Fluorinated tubes are used to stop glycolysis

- Self-Monitoring of Blood Glucose
  - People with diabetes should closely monitor their blood glucose levels to keep them as close to normal as possible.
  - Those with type 1 diabetes should check levels 3 or 4 times/day.
- Glucose Tolerance and 2-Hour Postprandial Tests
  - 2-hour postprandial test
    - Patient drinks standardized (75 g) glucose load.
    - Glucose measurement is taken 2 hours later.
  - Oral glucose tolerance test

The OGTT

The oral glucose tolerance test

Indications	Procedure
equivocal fasting/random blood glucose concentrations	patient should eat normal diet, containing at least 250 g carbohydrate per day for three days
unexplained glycosuria, particularly in pregnancy	fast patient overnight
clinical features of diabetes mellitus or its complications with normal blood glucose concentrations	take basal blood sample for glucose determination
diagnosis of acromegaly (see p. 145)	give 75 g glucose in water orally; take further blood sample at 120 min for glucose determination
	patient should rest throughout test; smoking not permitted; drinks of water are allowed

Role of Laboratory (cont'd)

- Glycosylated Hemoglobin/Hemoglobin A_1c
  - Long-term blood glucose regulation can be followed by measurement of glycosylated hemoglobin.
  - Provides clinician with time-averaged picture of patient's blood glucose concentration over past 6 – 8 weeks.
  - Glycosylated hemoglobin: formation of a hemoglobin compound produced when glucose reacts with amino group of hemoglobin
  - Hemoglobin A_1c (HbA_1c) is most commonly detected glycosylated hemoglobin.
  - Affinity chromatography is preferred method of measurement.

Pathophysiology & Clinical features of DM

- Two types: either:
  - metabolic disturbances
  - long-term complications

Metabolic complications of DM

May lead to coma . The causes can be ;

- Hyperglycemia
- Glycosuria: lead to osmotic diuresis, increased, water excretion (polyuria), increased plasma osmolality
- ●stimulate thirst center: polyuria + polydipsia
- Ketoacidosis
- Lactic acidosis.
- Hypoglycemia.
- Uraemia.

Diabetic Ketoacidosis (DKA)

- Insulin is inadequate due to infection ,trauma, unusal stress.
- Dehydration ,ketosis ,hyperventelation .
- Ketoacidosis may be due to ;

1. Insulin deficiency .
2. High regulatory hormones (adrenalin, cortisol, growth hormone ,glucagon.)
3. Hyperglcemia .

4-free faty acids .

5- osmotic diuresis causes loss of water Na ,K, Ca ,hypovolemia .

6- hyperkalemia.

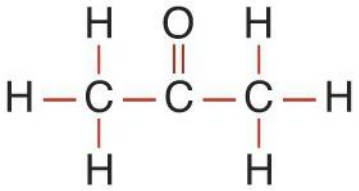
7-lactic acidosis ,uremia .

Role of Laboratory (cont'd)

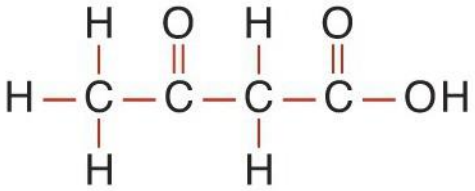
- Ketones
  - Produced by liver through metabolism of fatty acids
  - Provide a ready energy source from stored lipids
  - Increase with carbohydrate deprivation or decreased carbohydrate use (diabetes, starvation/fasting, high-fat diets)
  - Three ketone bodies
    - Acetone (2%)
    - Acetoacetic acid (20%)
    - 3-β-hydroxybutyric acid (78%)
  - Specimen requirement is *fresh* serum or urine.

Role of Laboratory (cont'd)

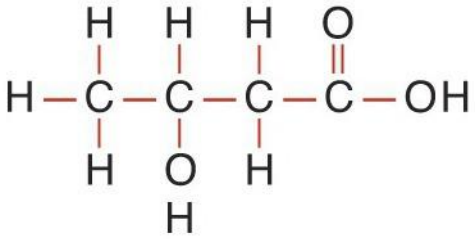
- Three ketone bodies



Acetone



Acetoacetic acid



β-Hydroxybutyric acid

Treatment

- K refluid replacement as isotonic saline.
- placement.
- $NaHCO_3$ (in sever cases only)
- Insulin is given.
- Dextrose is given later to maintain euglycemia
- Timing & repeat

Long-term complications of DM

- Occur in both types: 1 & 2. They are either:
  - microvascular complications:
    - *nephropathy
    - *neuropathy
    - *retinopathy
  - macrovascular disease:
    - * atherosclerosis

Role of Laboratory (cont'd)

- Microalbuminuria
  - Increase in urinary albumin is early sign of renal nephropathy, a complication of diabetes mellitus.
  - Annual assessment of kidney function by determination of urinary albumin excretion is recommended for diabetic patients.
  - Defined as persistent albuminuria in range of 30–299 mg/24 hr or albumin-creatinine ratio of 30–300 μg/mg
- Islet Autoantibody and Insulin Testing
  - Presence of autoantibodies to β-islet cells of pancreas is characteristic of type 1 diabetes.
  - Not currently recommended for diabetes diagnosis

Diabetic nephropathy

- It is the major cause of premature death in patients with DM (both types 1 & 2)
- The earliest detection of the abnormality is: **microalbuminuria**
- DM causes progressive changes to kidneys resulting ultimately in diabetic nephropathy
- It occur over years. It may be delayed by strong glycemic control
- Microalbumin measurements are useful to help in diagnosis at early stage
- For screening, random spot collection for the measurement of the albumin-to-creatinine ratio

Non-ketotic hyperglcemic coma

- Type II diabetes (hyperosmolar non-ketotic hyperglycemia)
- In severe hyperglycemia (>50 mmol/L) {900 mg/dL}
- Insulin deficiency ,older patients.
- Great loss of water &electrolytes .
- High osmolality ,dehydration .
- Oral hypoglycemic agents are giving .
- Increased thrombotic episodes . Anticoagulant is considerd.

Criteria for the diagnosis of DM

- Obesity
- Family history
- Race (membership of high risk population)
- History of GDM
- Hypertension
- Low HDL
- Elevated triglycerides conc.
- A history of impaired fasting glucose or impaired glucose tolerance

Hypoglycemia

- Decreased plasma glucose levels
- Can be transient & relatively insignificant or life-threatening
- Occurs in healthy-appearing and sick patients, as a result of reaction to medication or of illness
- Symptoms appear at glucose level of about 50–55 mg/dL.
- Symptoms: increased hunger, sweating, nausea & vomiting, dizziness, nervousness & shaking, blurred speech & sight, mental confusion

Hypoglycemia

- Blood glucose levels less than 2.2 mmol/L {40mg/dL}
- Two types of hypoglycemia:
    - fasting hypoglycemia
    - reactive (postprandial) hypoglycemia
- Warning signs and symptoms of hypoglycemia are all related to CNS
- For diagnosis: two stages in diagnosis of hypo:

1- confirmation of the low blood glucose level

2- and elucidation of the cause

Introduction to Biochemical Investigations in Clinical Medicine

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Alnoor University College

CLINICAL BIOCHEMISTRY

Lecture Notes

Simon Walker
Geoffrey Beckett
Peter Rae
Peter Ashby

9th Edition

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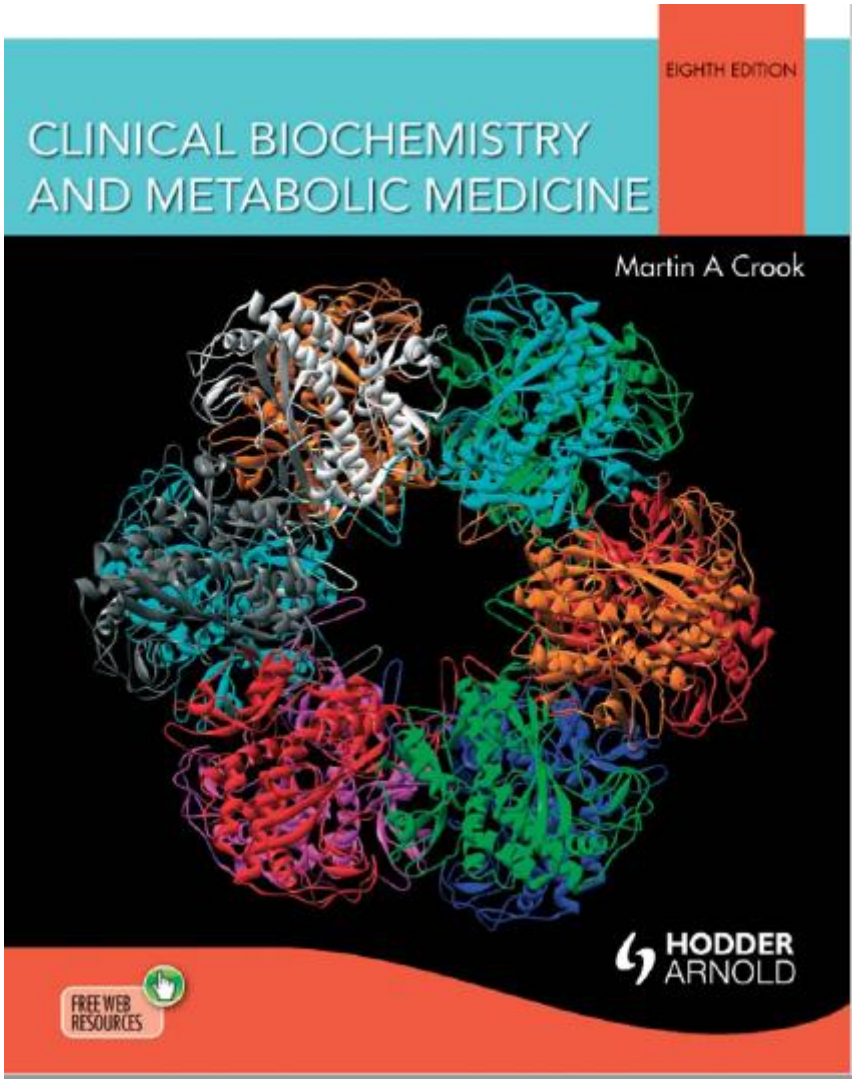
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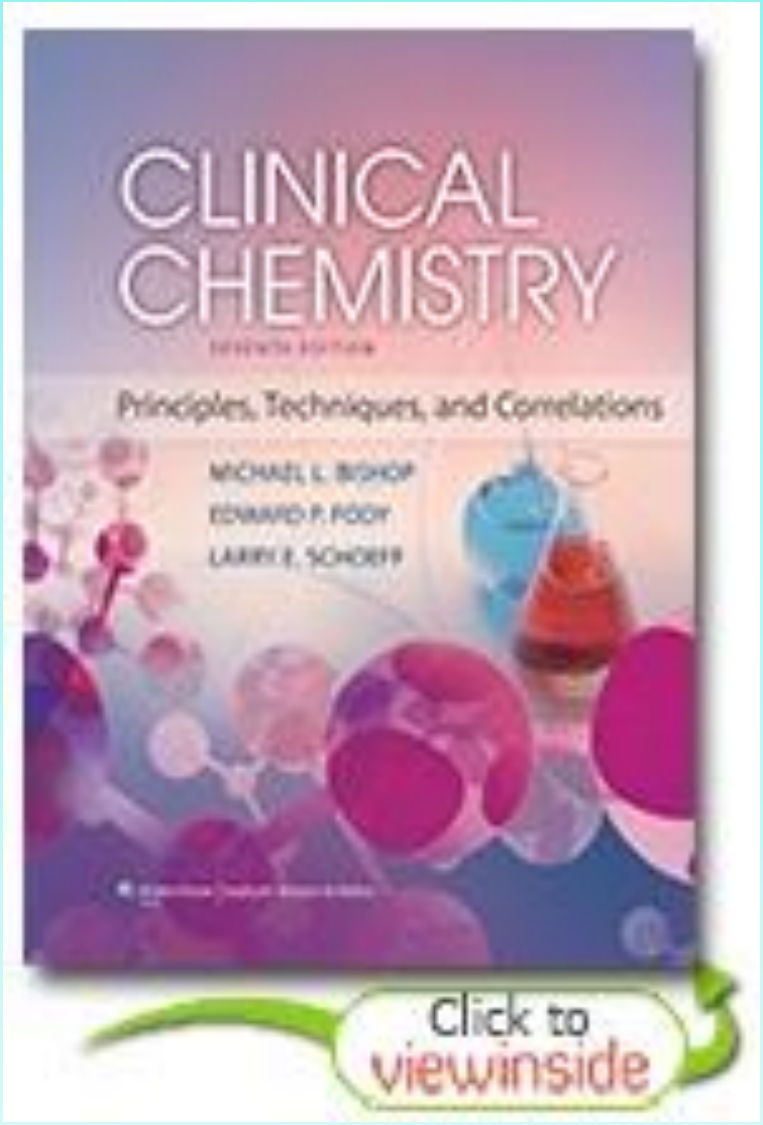
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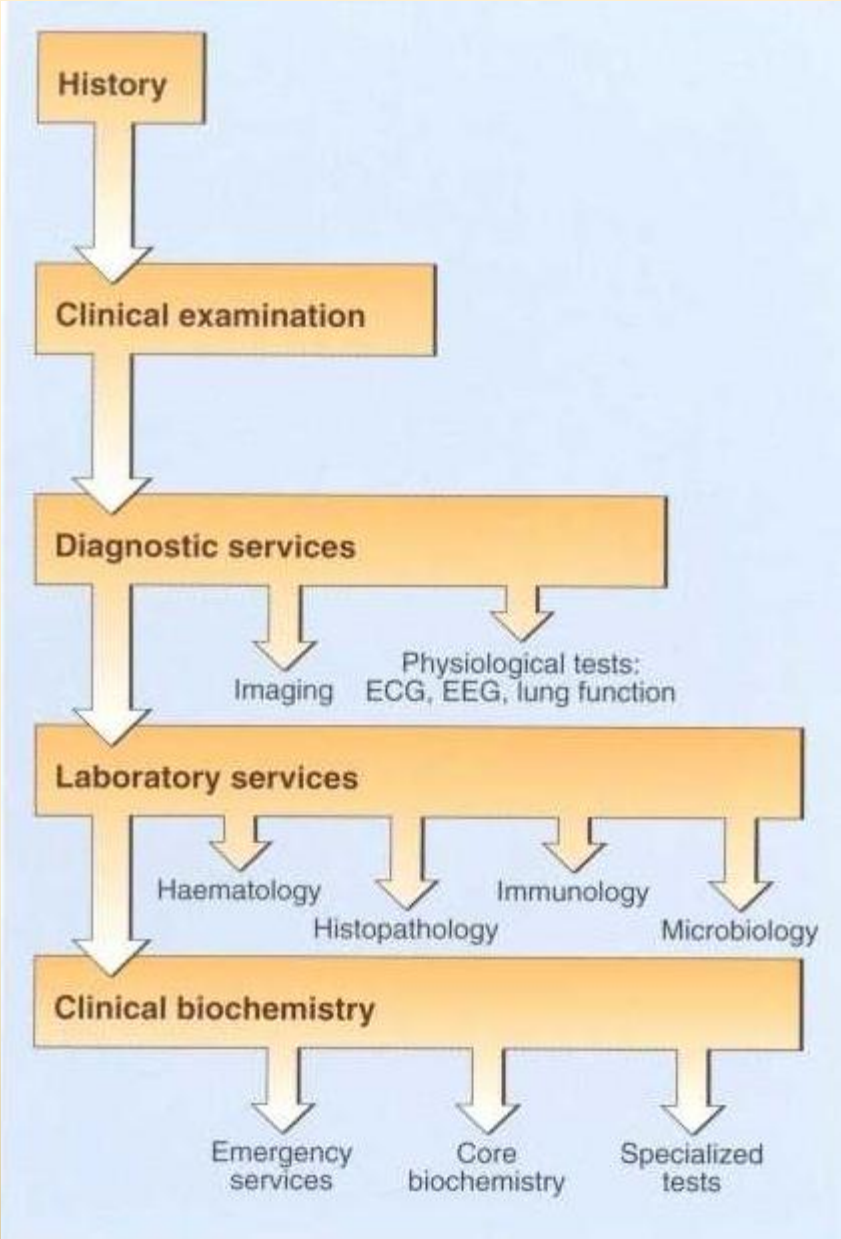
The place of clinical chemistry in medicine



Biochemical Investigation in Clinical Medicine

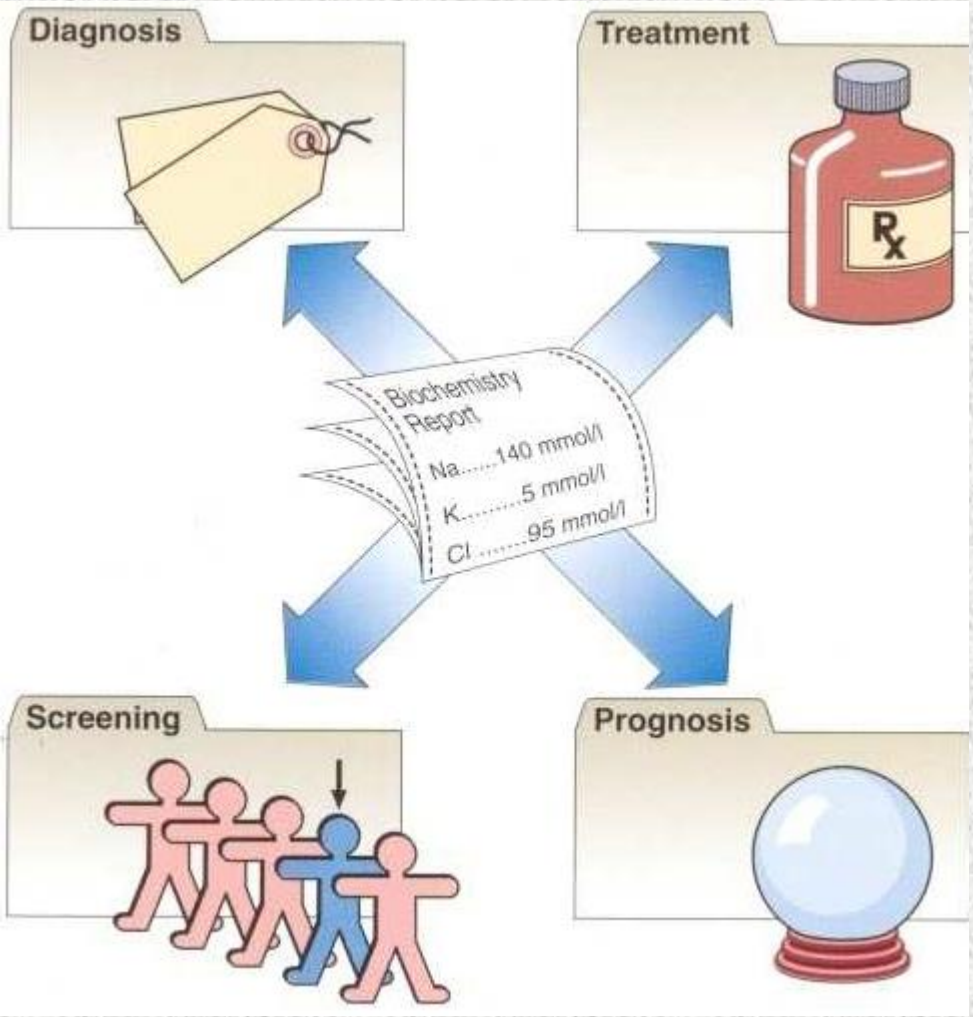
- to provide biochemical information for the management of patients
- such information will be of value only if it is

accurate and relevant

if its significance is appreciated by the clinician

so it can be used to guide to:

clinical decision-making



How biochemical tests are used

Collection of specimens

- Computer database showing patient identification data and history of lab. results
- Full labelling of test specimen:
- Patient’s name, sex, DOB and identification number
- Location, name of requesting doctor and time of sampling
- Name of the test and special notes

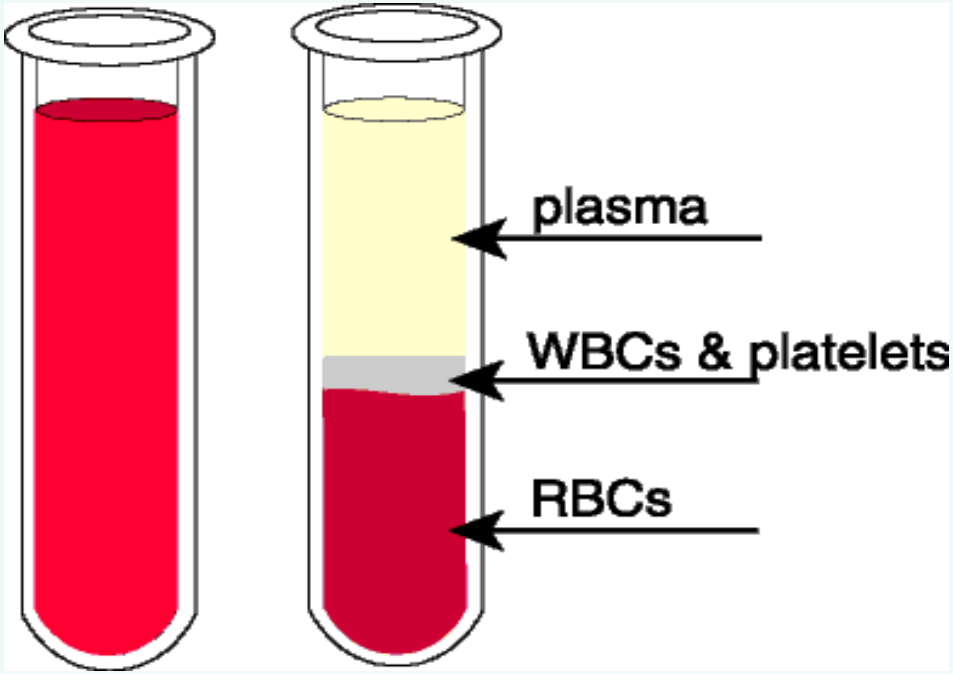
Specimens used for biochemical analyses

Venous blood, serum or plasma Arterial blood Capillary blood
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)

Biochemical test specimens

- Blood + anti-coagulant → centrifuge → aqueous supernatant → plasma

- Blood → clot → centrifuge → aqueous phase → serum



Sources of errors

- pre-analytical: occurring outside the lab e.g the wrong specimen being collected, mislabeling, incorrect preservation…

- analytical: occurring within the lab e.g human or instrumental error

- post-analytical: a correct result but is incorrectly recorded in the patient's record e.g: because of transcription error

Test selection (purposes of testing)

Category	Example
To confirm a diagnosis	Serum [free T4] and [thyroid-stimulating hormone, (TSH)] in suspected hyperthyroidism
To aid differential diagnosis	To distinguish between different forms of jaundice
To refine a diagnosis	Use of adrenocorticotrophic hormone (ACTH) to localise Cushing's syndrome
To assess the severity of disease	Serum [creatinine] or [urea] in renal disease
To monitor progress	Plasma [glucose] and serum [K^+] to follow treatment of patients with diabetic ketoacidosis (DKA)
To detect complications or side effects	Alanine aminotransferase (ALT) measurements in patients treated with hepatotoxic drugs
To monitor therapy	Serum drug concentrations in patients treated with anti-epileptic drugs

Point of care testing (POCT)

- Is defined as "those analytical patient-testing activities provided and performed outside the physical facilities of the clinical laboratories."
- Also named as "near-patient testing" .
- POCT bring the laboratory to the patient.
- POCT is increasingly being used in the:
  - emergency department
  - operating rooms
  - intensive care units (ICUs)
  - clinics
  - physician offices
  - nursing homes
  - pediatric units
  - pharmacies
  - ambulances

Point Of Care Testing (POCT)

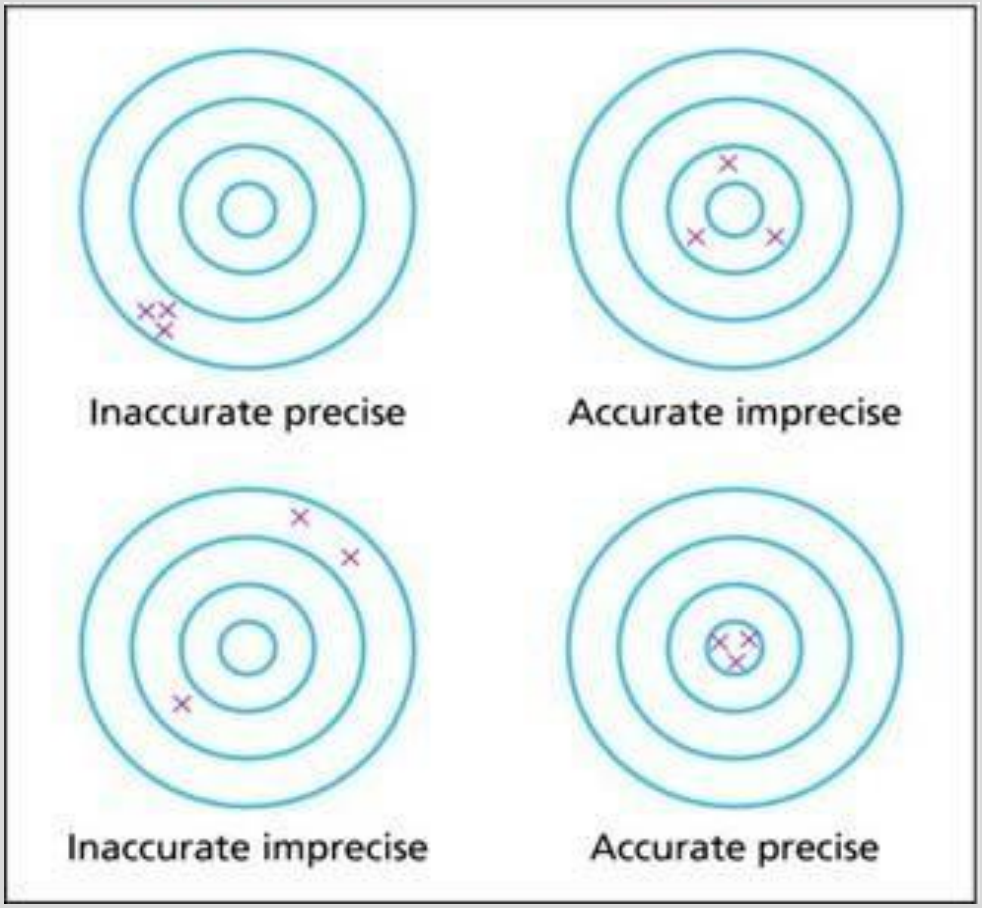
- Examples of POCT that are in common use:

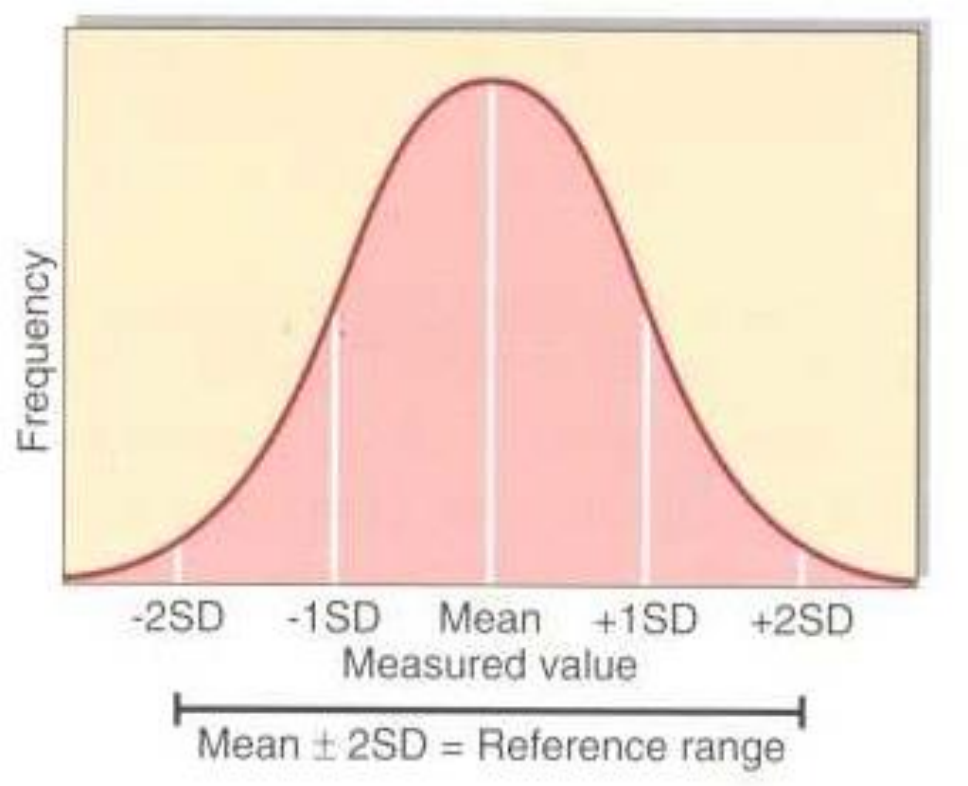
Common POCT in blood	Common POCT in urine
Blood gases	Glucose
Glucose	Ketones
Urea and creatinine	Red cells / Haemoglobin
Na, K and Ca	Bilirubin
Bilirubin	Urobilinogen
Salicylate	pH
Paracetamol	Protein
Alcohol	hCG (human Chorionic Gonadotrophin)
Troponin	Drugs of abuse

The ideal analytical method is:

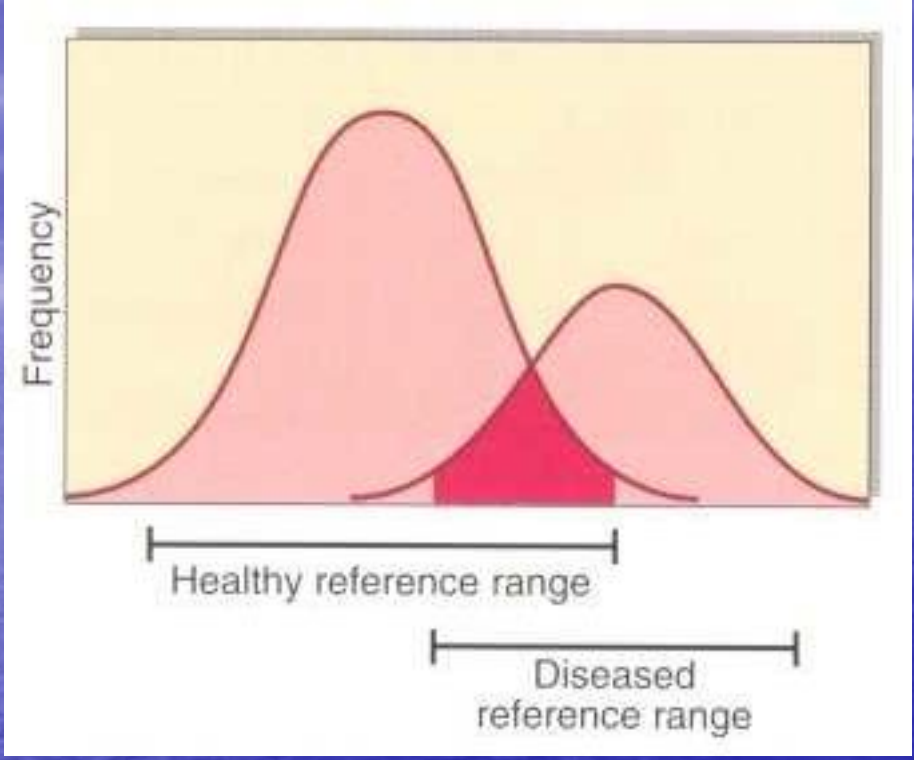
- accurate
- precise
- sensitive
- specific

- The ‘dartboard' analogy can be used to illustrate accuracy and precision.





Reference range in a normal healthy population



Overlap of biochemical results in health and disease

Interpretation of clinical chemistry results

- Normal or abnormal?
- Fit with previous assessment of this patient?
- Significant change than previous reports?
- Affect diagnosis?
- If I can’t explain results, then what do I propose to do about it?

Sources of variation

- Variability sources:

1- Analytical factors

2- Biological and pathological factors

1- Analytical sources of variation or error

- Consider the accuracy and precision
- Sensitivity and specificity
- Blunders

Biological sources of variation

A- Variable results in health

B- Variable results in disease (pathological source of variation)

- How do results vary in health?
- Within-individual variation (intra-individual variation)
- Between-individuals variation (inter-individual variation)

Biological sources of variation

A- Variable results in *health*

- Within-individual variation (intra-individual variation)

1- Diet

2- Time of day

3- Posture

4- Muscular exercise

5- Menstrual cycle

6- Drugs

Biological sources of variation

A- Variable results in ***health***

- Between-individual variation (inter-individual variation)

1- Age

2- Sex

3- Race

some important factors that influence biochemical variables

Factor	Example of variable affected
age	alkaline phosphatase, urate
sex	gonadal steroids
ethnicity	creatine kinase
pregnancy	urea
posture	proteins
exercise	creatine kinase
stress	prolactin
nutritional status	glucose
time	cortisol
drugs	triglycerides (alcohol) γ-glutamyl transferase (phenytoin)

- results can be compared either with reference range or with the results of previous tests
- an abnormal result does not always indicate the presence of a pathological process
- or a normal results does not always indicate the absence of a pathological process

الاستاذ الدكتور الصيدلاني

احمد يحيى احمد دلال باشي

استاذ في الكيمياء الحياتية الطبية

رئيس فرع الكيمياء الحياتية/ كلية الطب /سابقا

استاذ في قسم الصيدلة/ كلية النور الجامعة/ حاليا

الموضوع

الكيمياء السريرية العملي

Practical Clinical Chemistry

Types of specimens

- A human biological specimen is any material derived from a human, such as **blood, urine, tissues, organ, saliva , DNA/RNA, hair, nail clippings or any cells or fluids** whether collected for **research purposes** or for **diagnostic**, **therapeutic or surgical** procedures. The most important and often used is the **blood specimen**.
- **Reasons of tests:**
- - Diagnosis of diseases
- - Monitoring treatment.
- - Screening of diseases.
- - Research purposes

Blood

- Blood is a suspension of cells in a protein-salt matrix. The non-cellular portion of blood contains a series of proteins, some of which are involved in the coagulation process. This fluid is called **plasma.**
- When the coagulation process is allowed to proceed to completion, the non-cellular fluid, which can be separated from the clotted material, is termed **serum.**
- Blood used for biochemical analysis is collected from the veins, arteries or capillaries. For most testing, the site of phlebotomy has no analytical or physiological significance, so that venous blood is utilized because of ease of collection.
- For a limited number of analytes such as blood gases and lactate, significant difference arises between arterial and venous samples.

- Most testing is performed on the liquid or serum fraction of the blood that has been allowed to clot. The assumption is made that the distribution of constituents between cellular and extracellular compartments of blood is roughly equal.
- For some analytes it may be necessary to prevent the blood clotting process using an anticoagulant and the separated liquid (plasma) will be then used for analysis (see anticoagulants).
- Blood is usually drawn from a patient by a syringe, and transferred immediately to a clean plain tube (or a tube containing an anticoagulant) after removal of the needle to prevent breakdown of RBCs. The tube is then centrifuged after clot formation which takes about 10-15 min. (for serum) or immediately (for plasma).

Specimen collection:

- It is extremely important to collect, store and transport specimens correctly. Concerning blood collection, some factors should be considered before performing blood collection.
- 1- Diet: Dietary constituents may alter the concentration of analyst in blood significantly e.g. plasma glucose and triglyceride.
- 2- Drugs: for example significant hyperkalemia after few hours from taking potassium sparing diuretics.
- 3- Effect of posture: The concentrations of plasma proteins and of substances bound to them are lower if the patient is supine rather than standing.
- 4- I.V. Infusion.

special care when or during blood collection:

- 1- The skin must be clean over the site for collecting the blood specimen.
- 2- Venous stasis: It is usual to apply a tourniquet proximal to the site of venipuncture, if occlusion is maintained for more than a short time the combined effect of raised intra-capillary pressure and hypoxia of the vessel wall increases the rate of passage of water and small molecules from the lumen into the surrounding interstitial fluid.
- Large molecules, such as proteins and lipoproteins and R.B.C and other blood cells cannot pass through the capillary wall at the same time; their plasma concentrations therefore rise.
- In addition, the concentrations of plasma constituents bound to protein are increased. Therefore, prolonged venous stasis can raise the plasma total calcium concentration and other important protein-bound substances, including hormones and drugs.

- Moreover, prolonged stasis and hypoxia causes intracellular leakage of constituents, such as potassium and phosphate and result in a false high plasma concentration.
- 3- Site of venipuncture: if the patient is receiving I.V infusion the administered fluid dilute the blood, so blood taken from the opposite arm will give a valid result.
- 4- Posture: Should be standardized if possible, changing of posture from lying to standing suddenly may show an increase in concentration of plasma proteins by 13%.
- 5- Hemolysis: Should be avoided by using a suitable syringe needle.

- **Hemolysis**
- During sample collection and until the serum or plasma are isolated from RBCs, care must be taken to minimize the opportunities for hemolysis.
- Hemolysis may arise because of the use of too large or too small needle, moisture in a syringe, vigorous mixing of the blood, rapid expansion of the blood into the tube, or the separation process.
- Whatever the cause, hemolysis may interfere with a number of chemical procedures and should be avoided.
- Some constituents are present in high concentrations within the erythrocytes, and so hemolysis will falsely increase the value for those substances in serum such as potassium and enzymes (like glutamate oxaloacetate transaminase, lactate dehydrogenase and acid phosphatase).

- On the other hand, for those substances that exist at lower concentration in the red cells than outside, hemolysis will result in a dilution effect on the serum constituents such as sodium and chloride, so falsely low result will be obtained.
- In addition, hemoglobin may directly interfere in a chemical determination by inhibiting an enzyme such as lipase, by interfering with a reaction such as the reaction of diazo with bilirubin, or by yielding a significant color and thus interfering with a colorimetric analysis.
- Glucose changes most rapidly of all cell constituents when serum or plasma are left in contact with RBCs. Since glycolysis is an enzyme catalysed reaction, sodium flouride has to be added to the blood sample for glucose estimation to inhibit the enzyme enolase that is involved in glycolysis.

Anticoagulants

- 1- Heparin: α-mucoitin polysulphuric acid inhibits the formation of thrombin from prothrombin. It is usually available as the Na, K, NH_4 and Li salts.
- 2- EDTA (Ethylene diamine tetra acetate): It chelates calcium ions which are essential for clotting mechanism. Its dipotassium and dilithium salts are most often used.
- 3- Oxalate and citrate: Oxalate acts by precipitating the calcium. Potassium oxalate is the most soluble and so it is most commonly used. Sodium citrate does not precipitate calcium but converts it into a non-ionised form.
- 4- Sodium flouride: It is also considered as an anticoagulant, but since larger amounts are needed, it is rather used as a preservative for glucose determination by inhibiting red cell metabolism, glycolysis and bacterial action.

Urine

- **Collection of Urine Specimen;**
- Urine specimen tends to deteriorate unless the correct preservative is added from the start, or the specimen is refrigerated throughout the collection period.
- The changes in urine if not stored adequately include:
- 1- Destruction of glucose by bacteria.
- 2 - Conversion of urea into ammonia.
- 3- Oxidation of urobilinogen to urobilin.
- Different samples of urine specimen can be obtained such as random urine sample, and 24 hours urine sample.

- Single specimen of urine is used for general urine examination and for most qualitative tests. For quantitative work, 24 hour specimen is best employed,

24 hr urine collection

- For collecting a 24h sample, the patient empties the bladder first and the urine is discarded. All specimens passed thereafter during the day and during the following night are saved and the specimen obtained by emptying the bladder at the same time the following morning is added to them.
- The sample is collected in a clean covered container and kept in a cool place preferably in the refrigerator.
- If urine has to be kept, it may be necessary to prevent the effect of bacteria by adding a proper preservative such as hydrochloric acid, chloroform or formalin to the urine.

- **Other Body Fluids**
- The laboratory is also called upon to perform a variety of testing on **cerebrospinal fluid**, **amniotic fluid** and other **body fluids**. In all instances, it is essential to ensure that the proper specimen is collected and contamination is avoided.
- In addition, biochemical analysis may be performed on **stool** samples, **gastric aspiration**, and **renal** or **biliary calculi.**
- Stool samples are usually collected over 48-72 h periods.
- Renal calculi (stones) collection may be a random event depending on when the stone is passed.

BLOOD OR PLASMA GLUCOSE

Many analytical procedures have developed to measure blood glucose. Procedures in common use include enzymatic (glucose oxidase), colourimetric (O – toluidine) or oxidation reaction (copper sulphate).

Furthermore, whole blood was the sample of choice for analysis. However, values for glucose in whole blood are less than in plasma since red blood cells contain only about 80% water, compared with 93% in plasma, this despite the identical concentration of glucose in the water phase of both cells and plasma.

Therefore, glucose concentration in plasma is about 12% higher than in whole blood (depending on the haematocrit**).(haematocrit the proportion of red blood cells in the blood)**

Collection and handling of specimens

- When venous blood is drawn and permitted to clot, the average rate of decrease in serum glucose is approximately 7% in each hour (0.28 – 0.56 mmol/L) or 5-10 mg/dl. This decrease is the result of glycolysis.
- Serum in contact with RBCs without preservative must be separated from the cells or clot as soon as possible, as glucose values will decreases with time
- However, it is preferred to prevent reduction in blood glucose as a result of glycolysis by collecting blood into sodium fluoride containing tubes. Fluoride ions prevent glycolysis by inhibiting enolase enzyme.
- Cerebrospinal fluid (CSF) is frequently contaminated with bacteria or cellular constituents and should be analysed for glucose without delay or, otherwise, the sample should be preserved with sodium fluoride.

- Clinical significance
- Normal range (using enzymatic method)
- Plasma glucose 3.0 – 5.6 mmol/L (55-100 mg/dl)
- CSF glucose 2.2 – 4.2 mmol/L (40-75 mg/dl)
- or 60% of plasma value
- **Hyperglycaemia** (raised blood or plasma glucose) is the hallmark of diabetes mellitus. The common underlying defect is a deficiency of insulin action which may be absolute (as in type 1 diabetes) or relative with resistance to insulin action (as in type 2 diabetes).

- The ADA Diagnostic criteria of Diabetes (2010)

Parameter	normal	prediabetic	Diabetic
FPG (mg/dl)	<100	100-125	≥126
2-hr PG on OGTT (mg /dl)	<140	140-1 99	≥200
Random PG (mg /dl)	<140----------		≥200
HbA1c %	<5.7	5.7-6.4	≥6.5

- Oral glucose tolerance test (OGTT):
- OGTT is performed by giving the patient 75 g oral glucose dissolved in 250 – 300 ml water. Blood samples are taken before (fasting) and 2 hr. following the load for the measurement of glucose.
- A 2 hr. post glucose plasma glucose (2 hPG) level below 7.8 mmol/L (< 140 mg/dl) is considered as normal, higher than 11.1 mmol/L (≥ 200 mg/dl) is diagnostic of diabetes, while 2hPG between 7.8 – 11.1 mmol/L (140 – 199 mg/dl) is indicative of impaired glucose tolerance (IGT).

Glycated Hemoglobin (HbA1c):

Within the circulation sugars can bind non enzymically to haemoglobin.•
Binding of glucose to the N-terminal valine of the beta chains of HbA•
(the normal adult haemoglobin) forms a glycated haemoglobin called Hb A1c.•
Normally about 4-6% of the circulating haemoglobin is in this form.•
The life span of a red blood cell is about 12 weeks. Assuming normal red
cell life span, HbA1c thus reflects the average plasma glucose concentration.•
Normally, the hemoglobin consists of approximately 97% HbA, 2.5%•
HbA2 and 0.5% HbF. •
HbA consists of three different fractions: HbA1a, HbA1b and HbA1c. •
Collectively, these are referred to as HbA1c which reflect the average plasma glucose concentration over the preceding 12 weeks.

- **Hypoglycaemia**
- On the other hand, hypoglycaemia is defined as plasma glucose less than 2.5 mmol/L (45 mg/dl). It is less common than hyperglycaemia, and it is eventuated in practice in cases including insulinoma (pancreatic tumors), hepatoma (malignant tumor of the liver), adrenal carcinoma, hypopitutarism, addison's disease (when the body doesn't produce enough of certain hormones specialy cortisol and, often aldosterone), inborn error of metabolism of glycogen storage disease, galactossemia, fructose intolerance and also in essential reactive hypoglycaemia.

- **Enzymatic Method for Measurement of Glucose**
- **Principle: (Kit method) (BIOLABO)**
- Aldehyde group of glucose molecule is oxidized in the presence of **glucose oxidase (GOD)** to **gluconate** with the liberation of **hydrogen peroxide**, which in turn reacts with **phenol and 4-amino-antipyrine (4-aminophenazone)** under catalysis of **peroxidase** enzyme (POD) to form a pink quinoneimine dye. The absorbance of the colored complex is proportional to the concentration of glucose in the specimen and is measured at 500 nm.

- Glucose + O2 + H2O → Gluconate + H2O2
-
- 2H2O2 + phenol + 4-amino-antipyrine —Peroxidase→ Quinoneimine + 4H2O (pink dye)
- **Procedure (Kit method) (BIOLABO)**
- Pipette into test tubes

	Blank	Std	Test
Working reagent (ml)	1	1	1
Standard/R3 (ml)	-	0.01	-
Sample (serum) (ml)	-	-	0.01
Distilled water (ml)	0.01	-	-

- *Note: Working solution is made by adding contents of vial R1, into vial R2 (R1 is the buffer solution, (GOD), (POD) and Amino-antipyrine. R2 is the is the phenol .*

- Mix, incubate at 37°C for 10 minutes or for 20 minutes at room temperature (25°C). Read absorbance of sample against reagent blank within 20 minutes at 500 nm.

- **Calculation**

- Glucose concentration = $\frac{A_t - A_B}{A_{st} - A_B}$ x 100 mg/dl (5.55 mmol/L)

Reagents

- **Kit enzymatic method**
- **Contents:**

1. Reagent/R1: (buffer reagent)

• Phosphate buffer, pH 7.5	150 mmol/L
• Glucose oxidase (GOD)	20000 UI/L
• Peroxidase (POD)	1000 UI/L
• 4-Amino-antipyrine (PAP)	0.8 mmol/L

2. Reagent/R2:

Cloro-4-phenol	2 mmol/L

3. Reagent/R3: (standard)

• Glucose	100 mg /dl (5.55 mmol/L)

- **Working reagent** is prepared by adding contents of vial (R1) into vial (R2). Mix gently until complete dissolution.

- **Vial R3: Ready to use**

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Measurement of serum Urea

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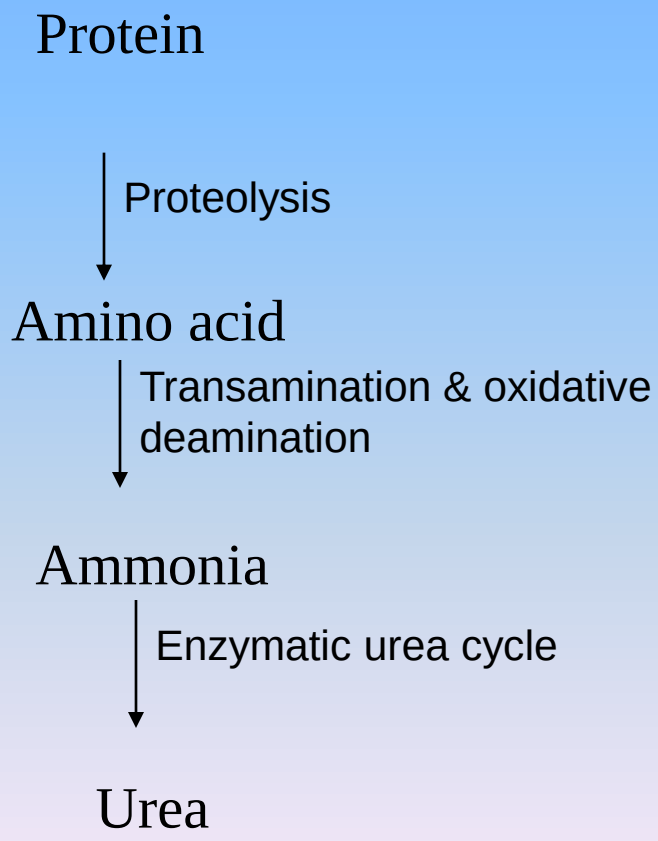
UREA

Urea is the major nitrogen metabolic product of protein catabolism.

The biosynthesis of urea from ammonia is carried out exclusively by hepatic enzymes of the urea cycle



Urea is the major end product of amino acid catabolism, it is synthesized in the liver and most of urea is execrated in the urine (90%) .

So, urea is normally present in the in blood or serum . The normal value is around 20 mg% (3.3 mmol/l). The normal range is between 20-40 mg% (3.3-6.6 mmol/l).

When higher values are detected it is regarded abnormal and may be due to kidney dysfunction, excessive breakdown of protein, dehydration (or diuretic treatment) or heart diseases.

In renal disorders determination of serum urea is always associated with that of creatinine, as high serum urea levels are not specific to renal disorders and can also be observed in cases of protein-rich diets.

Principle:(Kit method BioSystems)

Urease hydrolyses urea by producing ammonium ion.

$$Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$$

In alkaline medium, the ammonium ions react with salicylate and hypochlorite to form a green colored indophenols (2,2-dicarboxyl indophenols).

The reaction is catalyzed by sodium nitroprosside.

NH_4^+ + Salicylate + Hypochlorite $\xrightarrow[\text{Sod. Nitroprosside}]{\text{Alkaline medium}}$ Indophenols (Green color)

Reagents:

A1 Reagent: (Color reagent)

Sodium salicylate 62 mmol/L

Sodium nitroprusside 3.4 mmol/L

Phosphate buffer 20 mmol/L pH 6.9

A2 Reagent: Urease 500U/ml

R1 (Alkaline reagent):

Sodium hypochlorite 7 mmol/L

Sodium hydroxide 150 mmol/L

R2 (Standard Reagent): Standard urea 50 mg/dl (8.3 mmol/L)

R3 (Working reagent) : Transfer the contents of reagent A2 vial into reagent A1 bottle. Mix thoroughly (or 1 ml A2+24 ml A1)

Procedure: (Kit method BioSystems)

In three test tubes make the following additions:

	St	Test	Blank
Standard solution (**R2**)	10 µl	--	--
Sample (Serum)	--	10µl	--
Distilled water	--	--	10µl
Working solution(**R3**) (A2+A1)	1ml	1 ml	1 ml

(A1 Coloring reagents (Sodium Salicylate + Sodium Nitroprosside + Phosphate Buffer) + A2 Urease)

Mix and incubate for 10 minutes at 20-25 °C or 5 minutes at 37 °C

Alkaline Solution (**R1**)	1 ml	1 ml	1 ml

{**R1**: Alkaline reagent (Sodium hypochlorite + Sodium Hydroxide)}

Mix and incubate for 10 Minutes at 20-25 °C. or 5 minutes at 37 °C

Measure the absorbance at 600 nm.

Calculation:

$$\text{Serum Conc. Of urea} = \frac{A_T - A_B}{A_{St} - A_B} \times \text{Conc. Of St}$$

(50 mg%)
(or 8.33 mmol/l)

Conversion factor:

mmol/l X 6 = mg%

mg% X 0.167 = mmol/l

- **URIC ACID**
- Uric acid is the end product of purine metabolism. It is a waste product derived from purines of the diet and those synthesized in the body. In human, uric acid arises from ingested nucleoproteins, degradation of nucleoproteins, in nuclear material and by synthesis from simple precursors.
- Healthy adult human body contains about 1.1 g of uric acid. Normally about one half of uric acid is eliminated and replaced each day, partly by urinary excretion and partly through destruction in the intestinal tract by microorganisms. Serum uric acid is freely filtered by the glomeruli (98-100%).
- Uric acid concentration in serum is greatly affected by extrarenal as well as renal factors. Its concentration depends upon the net balance achieved between the rate of synthesis of purines or breakdown of nucleoproteins on one hand, and the rate of elimination of uric acid on the other.

Clinical significance

- Normal levels: Males 0.21 – 0.42 mmol/L (3.5 – 7.0 mg/dl)
- Females 0.15 – 0.36 mmol/L (2.6 – 6.0 mg/dl)
- In the presence of normally functioning kidney, the ingestion of nucleoproteins has little influence on serum uric acid unless very large amounts are taken.
- Hyperuricaemia is most commonly defined by serum uric acid concentration over 0.42 mmol/l (7.0 mg/dl) in men, or over 0.35 mmol/l (6.0 mg/dl) in women.

The major causes of hyperuricaemia are:-

- 1. Essential hyperuricaemia (gout).
- 2. Impaired renal excretion: Renal failure, drug therapy e.g.
- diuretics, alcohol, ketoacidosis.
- 3. Increased nucleic acids turnover: Myeloproliferative disorders
- e.g. leukaemia, polycythemia.
- (a group of diseases in which the bone marrow makes too many red blood cells, white blood cells, or platelets)
- 4. Specific enzyme defect: Deficiency of HGPRT enzyme (hypoxanthine-guanine phosphoribosyl transferase) in Lesch-Nyhan syndrome.(**one of the central enzymes that recycle the building blocks of RNA and DNA)**

Principle (Kit method by BIOLABO)

- Uricase (an enzyme act on uric acid) act on uric acid to produce **allantoin, carbon dioxide and hydrogen peroxide**.
- In the presence of **peroxidase, hydrogen peroxide react with chromogen** (4-amino-antipyrine and dichloro-hyroxybenzene sulfonate) to yield **quinoneimine** (a red color complex).
- The absorbance is measured at 520 nm is proportional to the amount of uric acid in the specimen.
- Uric Acid $\xrightarrow{\text{Uricase}}$ Allantoin +CO_2 + H_2O_2
- H_2O_2 + Chromogen $\xrightarrow{\text{Peroxidase}}$ quinoneimine (red color complex)
- **(chromogen =** Amino-antipyrine + Dichlorohyroxybenzene sulfonate)

Reagents (Kit method by BIOLABO)

- **R1: Buffer pH 8**
- Dichloro-hyroxybenzene sulfonate 3 mmol/L
- Potassium hexacyano ferrate 53 µmol/L
- 3-DDAPS 0.7 mmol/L
- EDTA 2 mmol/L
- **R2: Enzymes**
- Peroxidase 2000U/L
- Amino-antipyrine 750 µmol/L
- Uricase 500 U/L
- **R3: Standard** Uric acid 10 mg/dl

Procedure: (Kit method by BIOLABO)

- Prepare 3 clean and dry test tubes, label them as T, S, & B. Make the following additions:

	T	S	B
R1: Buffer (Dichloro-hyroxybenzene sulfonate +Potassium hexacyano ferrate)	800 µl	800 µl	800 µl
R3: Standard	-	25 µl	-
Sample (serum)	25 µl	-	-
Distilled water	-	-	25 µl
R2 : Enzymes (Peroxidase & Uricase and 4- Amino-antipyrine)	200 µl	200 µl	200 µl

- ----------

Mix and incubate for 10 Minutes at 20-25 °C or 5 minutes at 37 °C
Measure the absorbance at 505 nm.

Calculation:

$$\text{Serum Conc. of uric acid} = \frac{A_T - A_B}{A_{St} - A_B} \times \text{Conc. of St (10 mg/dl)}$$

SERUM TOTAL CALCIUM

Calcium is the most abundant mineral in the body, there being about 1 kg (25000 mmol) calcium in the body of which 99% is present in the bones. The remaining 1% circulates in blood in two main forms, the albumin bound fraction which accounts for 50% of the total blood calcium and it is physiologically inactive.

The other part is the free ionized calcium which is physiologically active. The free ionized calcium is essential for coagulation process, neuromuscular activity, membrane permeability, and the activity of many enzymes. Ionized calcium is also very important inside the cell where it acts as a second messenger for many hormones.

Second messengers are intracellular molecules do certain physiological changes such as proliferation, migration etc…. The cell releases second messenger molecules in response to exposure to extracellular signaling molecules (first messengers).

First messengers are extracellular factors, often hormones or neurotransmitters that contain peptides, which are biochemically hydrophilic molecules, these first messengers may not physically cross the phospholipid bilayer to initiate changes within the cell directly.

- There are many factors that control the level of circulating free ionized calcium which include:-
- 1- Parathyroid hormone.
- 2- 1,25 dihyroxy cholecalciferol, 1,25 DHCC (calcitriol).
- 3- Calcitonin hormone.
- In the lab, we measure the total calcium of the blood (free + bound).

Clinical significance

- Normal range: 2.10 – 2.60 mmol/L (8.5-10.5 mg/dl).

Serum total calcium increases in hyperparathyroidism, malignancy with metastases, multiple myeloma (bone marrow cancer) and vitamin D intoxication.

Serum total calcium may be increased due to excessive venous stasis during blood collection and therefore correction for albumin or total protein has to be made.(To make use of correction factor: For each g/L that the plasma [albumin] is above or below g/L , 0.02 mmol/L should be subtracted or added respectively from the value for plasma [calcium] observed in the same specimen).

- Serum total calcium decreases in hypoparathyroidism, osteomalacia, rickets, chronic renal failure, malabsorption syndrome and hypoproteinemia.

Determination of Calcium
(Kit method) (BIOLABO)

- **Principle**
- In alkaline solution CPC (O-cresolphthalein complexone) reacts with calcium to form a dark-red colored complex .
- The absorbance of this colour is measured at 570 nm that is proportional to the amount of calcium in the specimen.
- Note: interference due to Mg^{+2} ion is eliminated by 8- hydroxyquinoline.

Procedure: (Kit method)(BIOLABO)

	Test	Standard	Blank
Serum	25 µl	---	---
Standard 10 mg/dl (2.5 mmol/L)	---	25 µl	---
Distilled water	---	---	25 µl
working solution (R1+R2)	1 ml	1 ml	1ml

R1: (Amino-2-methyl-popanol-1+ Hydrochloric acid) + R2: (O-cresolphtalein complexone (CPC) + Hydroxy-8-Quinoline + Hydrochloric acid)

Mix well incubate for 5 minutes at room temperature.
Read absorbance at 570 nm against blank.
The colour is stable for 1 hour away from light.

Calculation

- Total Serum Calcium = $\frac{A_t - A_B}{A_{st} - A_B}$ x Standard Concentation
- Total Serum Calcium = $\frac{A_t - A_B}{A_{st} - A_B}$ x 10 mg/dl (2.5 mmol/L)
  Concentration
- Normal range: 2.10 – 2.60 mmol/L or
- (8.5-10.5 mg/dl).

Reagents

- **R1 Calcium Buffer**
- Amino-2-methyl-popanol-1 1.7 mmol/L pH 11
- Hydrochloric acid 210 mmol/L
- **R2 Calcium dye**
- O-cresolphtalein complexone (CPC) 78 miromol/L
- Hydroxy-8-Quinoline 3.36 mmol/L
- Hydrochloric acid 25 mmol/L
- **R3 Calcium standard**
- Calcium 10 mg/dl (2.5mmol/L)
- **Working solution**
- Mix 1 volume **R1** and 1 volume **E2.** Reagents may also be added separatly.

SERUM INORGANIC PHOSPHORUS

Phosphate is an important anion associated with calcium in vivo. Knowledge of its plasma level is needed to interpret disturbances of calcium metabolism. The cellular 'high-energy' compounds like ATP, GTP are of great biological importance.

Clinical significance

There is often a marked variation in plasma phosphate concentration during the day, specially following meals. Reference values relate to specimens collected under fasting conditions vary significantly with age; being higher during infancy and childhood.

Fasting serum inorganic phosphate normal range of adults is 0.8-1.5 mmol/l (2.4-4.5 mg/dl) and in children it is 1.29-2.25 mmol/L (4-7 mg/dl).

Abnormalities of Serum Phosphate Concentration

- 1- Hypophosphatemia usually due to:
- a- Disturbances of calcium metabolism as a consequence of excess circulating PTH, as in primary and tertiary hyperparathyroidism.
- b- Renal disorders of phosphate reabsorption (phosphate lost from the body), as in renal tubular acidosis.
- c- During parenteral nutrition with inadequate phosphate and following intravenous glucose therapy.
- d- Chronic or long standing intestinal malabsorption.

- 2- **Hyperphosphatemia**
- a- Renal glomerular failure, as in chronic renal failure (CRF).
- b- Hypoparathyroidism.
- c- Catabolic states.
- d- Vit. D intoxication.

Principle: (Kit method) (BIOLABO)

- In an **acid medium**, **phosphate ions reacts** with **ammonium molybdate to** form **phosphomolybdic complex**.
- The absorbance of phosphomolybdic complex is measured at 340 nm that is proportional to the concentration of phosphate ions in the specimen.

- Phosphate + ammonium $\xrightarrow{\text{Acid medium}}$ phosphomolybdic complex
- ions molybdate

Procedure: (Kit method) (BIOLABO)

	Blank	Standared	Test
Reagent	1 ml	1 ml	1 ml
D. Water	20 µl		
Standard	---	20 µl	---
Specimen	---	---	20 µl

(Reagent: Ammonium molybdate + Sulfuric acid)

Mix well, incubate for 2 minutes at room temperature.

Read standard and test absorbances at 340nm against reagent blank.

Calculation::

Serum inorganic phosphorus = $\frac{A_T - A_B}{A_{std} - A_B}$ X Std Conc

Serum inorganic phosphorus = $\frac{A_T - A_B}{A_{std} - A_B}$ X 5 mg/dl (1.62 mmol/L)

Normal range (Adults): 0.8-1.5 mmol/l (2.4-4.5 mg/dl)

Children: 1.29-2.25 mmol/L (4-7 mg/dl).

Reagents

R1 pH Molybdate reagent

Ammonium molybdate 0.63 mmol/L

Sulfuric acid 210 mmol/L

Surfactant

R2 Standard inorganic phosphate 5 mg/dl (1.62 mmol/L)

SERUM TOTAL CHOLESTEROL

It is generally agreed that the normal range for total cholesterol is rather wide. Serum total cholesterol is slightly higher in men than in women. It is a little lower in persons under 20 years but rises with age. It does not appear to be much altered following meals (unlike triglyceride).

In pregnancy there is an increase which may reach 20% above normal at the 30th week.

Recently, for the lipid pattern to be clear, the lipid profile is used as an index of its possible effect in precipitating atherosclerosis. The following table is usually used for its interpretation:

- Serum total cholesterol < 5 mmol/L (<200 mg%)
- HDL-Cholesterol (Low risk) >1.4mmol/L (>55 mg%)
- (High risk) <0.9 mmol/L (<35 mg%)
- LDL-Cholesterol (Low risk) <3.4 mmol/L (<130 mg%)
- (High risk) >4.1 mmol/L (>160 mg%)
- VLDL-Cholesterol (Low risk) <.45 mmol/L (<37 mg%)
- Atherogenic index (Low risk) < 5 mmol/L (>6 High risk)
- Serum Phospholipids 57 – 89 mmol/L (175 – 275 mg%)
- Serum Triglyceride male 0.5 – 2 mmol/L (44 – 185 mg%)
- Female 0.4- 1.5 mmol/L (35 – 130 mg%)

Hypercholesterolemia

- Increases are found most characteristically in the primary hypercholesterolaemia (particularly types II, III and IV)**(elevated levels of cholesterol products, especially LDL and apoB),** nephrotic syndrome, hypothyroidism, obstructive jaundice, Primary biliary cholangitis, (previously called primary biliary cirrhosis), **(chronic disease in which the bile ducts in the liver are slowly destroyed)** and diabetes mellitus.
- Xanthomatosis is frequently associated with an increase in serum cholesterol. Primary xanthomatosis is divided into two groups, in one of which there is raised serum cholesterol, whereas in the other it is within normal limits, the deposits being composed of other lipids (Triglyceride).
- In secondary xanthomatosis the condition accompanies hypercholesterolaemia arising from one of the conditions listed above.
- Raised serum cholesterol signifies hypercholesterolaemia which is considered to be a cardiovascular risk factor that may predispose to coronary thrombosis appearing as angina pectoris or myocardial infarction.

Hypocholesterolemia

- Decreases are not so well defined. Conditions that may create hypocholesterolaemia include hyperthyroidism, malabsorption syndrome, malnutrition and pernicious anaemia **(an autoimmune condition that affects the stomach)**. Very low values occur in a beta lipoproteinaemia. Therapeutic reduction of serum cholesterol is seen during administration of lipid lowering drugs such as statin derivatives, clofibrate, cholestyramine and nicotinic acid.

Principle: (Kit method) (BIOLABO)

Serum cholesterol is measured by the following enzymatic reactions;

Cholesterol esters+ H_2O $\xrightarrow[\text{esterase}]{\text{Cholesterol}}$ Cholesterol + Fatty acids

- Cholesterol+O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ 4-Cholesten-3-One+H_2O_2

- $2H_2O_2$+4-aminoantipyrine+Phenol $\xrightarrow{\text{Peroxidase}}$ Red quinone + 4 H_2O

- By measuring the absorbency of the red quinone at 500 nm and comparing with standard solution of cholesterol treated in the same way and applying lamberts and Beers low , the concentration of the test can be found.

Procedure: (Kit method) (BIOLABO)

- Prepare three test tubes and label them as T, S, and B for test, standard and blank respectively and make the following additions:

	T	S	B
• Working solution (Which is made of R1+R2)(i.e. containing esterase, oxidase and peroxidase in addition to the 4-aminoantipyrine and phenol)	1ml	1ml	1ml
• Serum	10µl	-	-
• Standard Cholesterol	-	10µl	-
• Distilled Water	-	-	10µl

- _____
- Mix and incubate for 5 minutes at 37ºC or for 10 minutes at room temperature then apply the following calculations.
-

Calculation:

- mg% of Serum Cholesterol = $\frac{\text{At-AB}}{\text{Ast-AB}}$ X Conc. Of standard

$$\frac{\text{At-AB}}{\text{Ast-AB}} \times 200 \text{ mg/dl}$$

- Normal range for Serum total cholesterol= < 5 mmol/L (<200 mg%)

•Conversion factor:

- mmol / L = mg% X 0.0259

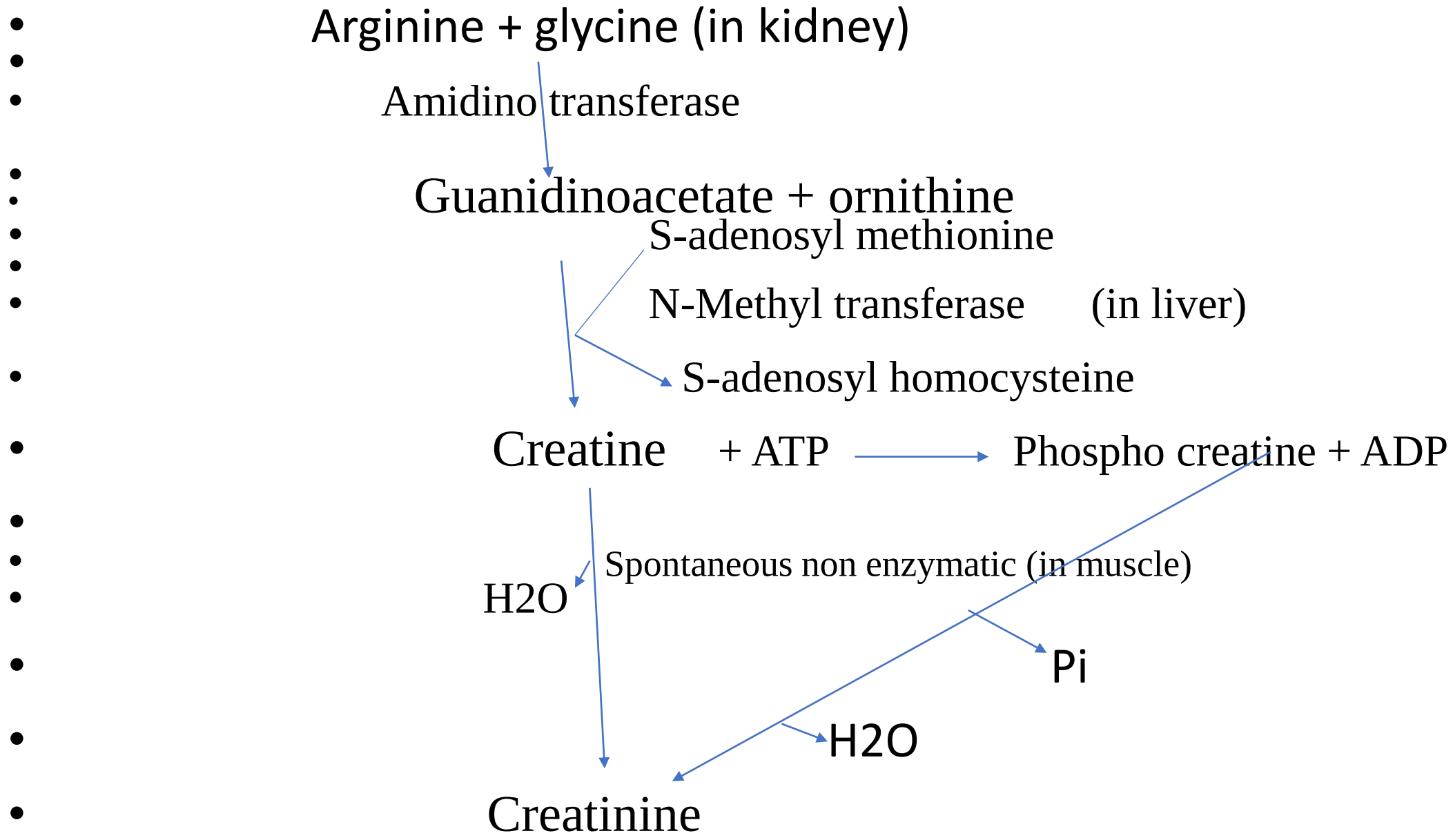
Reagents

- **R1 : Enzymes** (Cholesterol estrase, Cholesterol oxidase and
- Peroxidase.
- **R2** : 4-aminoantipyrine, Phenol and Buffer
- **R3** : Standard (200 mg/dl)
- **Working reagent** (Mix R1 and R2) so it contain:
- Phosphate buffer 100 mmol/L
- Chloro-4-Phenol 5 mmol/L
- Sodium Chlorate 2.3 mmol/L
- Triton X 100 1.5 mmol/L
- Cholesterol oxidase 100 IU/L
- Cholesterol esterase 170 IU/L
- Peroxidase 1200 IU/L
- 4-Amino Antipyrin(PAP) 0.25 mmol/L

SERUM CREATININE AND CREATININE CLEARANCE

Creatinine is synthesized in the kidney, liver and pancreas by two enzymatically mediated reactions. In the first, transamination of arginine and glycine to form guanidinoacetic acid. The second, methylation of guanidinoacetic acid occurs with S-adenosyl methionine as methyl donor to from creatine.

Creatine is then transported to body organs such as muscle and brain where it is phosphorylated to phosphocreatine, a high energy compound. Interconversion of phosphocreatine and creatine is a particular feature of metabolic processes of muscle contraction with some of the free creatine in muscle is spontaneously converted to creatinine (it’s anhydrous form).


Arginine + glycine (in kidney)
Amidino transferase
Guanidinoacetate + ornithine
S-adenosyl methionine
N-Methyl transferase
(in liver)
S-adenosyl homocysteine
Creatine
+ ATP
Phospho creatine + ADP
Spontaneous non enzymatic (in muscle)
H2O
Pi
H2O
Creatinine

- About 1-2% of muscle creatine is converted to creatinine daily. Because the amount of endogenous creatinine produced is proportional to muscle mass, the production varies with age, sex and body mass.
- Non-obese adult male excretes about 1.5 g/day, females 1.2 g/day.
- Creatinine is excreted mainly by the kidney. Following filtration, no further reabsorption of creatinine will occur through the tubules. As a result, creatinine clearance represents an indicator of glomerular filtration rate (GFR).
- Creatinine has a constant range. Its measurement is used to evaluate renal function (particularly glomerular). However with mild renal impairment, plasma creatinine remains fairly unchanged until GFR is decreased to 50-60% of its normal value.
- Therefore, an increased plasma creatinine indicates marked impairment of renal function and for the assessment of mild impairment, calculation of creatinine clearance is required. (a more sensitive index of renal function).

Endogenous creatinine clearance

- Renal clearance of a substance (creatinine in this case) is a figure (ml/min) representing the volume of blood from which the compound is completely cleared each minute by the kidney.
- **Instructions and method**
- 1- Hydrate the patient properly with at least 600 ml of water.
- 2- Have the patient void and discard the urine.
- Note: Record the time, and from then on collect all urine passed for usually 24 hours.
- 3- Collect a blood specimen preferable at mid time of urine collection.
- 4- In the laboratory, measure the volume of total urine collected and record both volume and minutes (hx60) of the period in which it was collected. Perform the assays of serum (or plasma) and urine creatinine.

5. Calculate clearance

$$\text{Cr. clearance (ml/min)} = \frac{U_{cr} \times V}{P_{cr}}$$

- Where Ucr = urine creatinine concentration
- Pcr = plasma creatinine concentration, in the same units.
- V = volume of urine flow in ml/min, calculated by dividing
- urine volume by collecting time (h x 60).
- 6- For children, obese and elderly patients measured clearance should be corrected for the surface area
- Corrected clearance = measured clearance X 1.73/ A
- Where A = surface area in square meter, 1.73 average adult's surface area. The surface area can be obtained from knowing the length and weight of the patient.

Clinical significance

- An increase in plasma creatinine is likely to be due to a fall in the GFR, the causes of which include:
- 1- Any disease in which there is impaired renal perfusion. (e.g. reduced blood pressure, fluid depletion, renal artery stenosis).
- 2- Most diseases in which there is loss of functioning nephrons (e.g. urinary tract obstruction due to prostatic enlargement).
- **Normal range**
- Serum or plasma creatinine – M= 62-115 μmol/L (0.7-1.3 mg/dl)
- F= 53-97 μmol/L (0.6-1.1 mg/dl)

- Urine creatinine – M= 7 - 14 mmol/24 hours (0.6 and 1.2 mg/dl)
- F= 6 - 13 mmol/24 hours (0.5 and 1.1 mg/dl)

- Creatinine clearance – M= 97 to 137 mL/min (1.65 to 2.33 mL/s)
- F = 88 to 128 mL/min (14.96 to 2.18 mL/s)

Principle: (Kit method)(BIOLABO)

- Colourimetric reaction (Jaffe reaction) of creatinine with alkaline picreate resulting in an orange colour. The absorbance of this colour is measured at 490 nm.is proportional to creatinine in the specimen.

- Creatinine + Picric Acid $\xrightarrow[\text{medium}]{\text{Alkaline}}$ Creatinine picrate
- Orange colour

Procedure: Kinetic method (BIOLABO)

- Let stand reagent and specimen at room temperature

	Test	Standared	Blank
Working reagent (R1+R2)	1000 µl	1000 µl	1000 µl
Specimen (Serum)	100 µl	---	---
Standard	---	100 µl	---
Distilled water	---	---	100 µl

Mix well. Incubate at 37°C for 3 minutes. Read absorbance A at 490 nm.

Working solution: R1 **(**Disodium phosphate + Sodium hydroxide) +
R2 **(**Sodium dodecyl sulfate + Picric acid)

Calculation:

Serum creatinine in mg/dl = $\frac{A_T - A_B}{A_{Std} - A_B}$ X Conc. of std. 2 mg/dl (177 µmol/L)

Normal range : M= 62-115 µmol/L (0.7-1.3 mg/dl)

- F= 53-97 µmol/L (0.6-1.1 mg/dl)

Urine creatinine in mg/dl = $\frac{A_T - A_B}{A_{Std} - A_B}$ X Conc. of std. 2 mg/dl (177 µmol/L) X dilution factor

- Normal range : M= 7 - 14 mmol/24 hours (0.6 and 1.2 mg/dl)
- F= 6 - 13 mmol/24 hours (0.5 and 1.1 mg/dl)

Creatinine clearance ml/minute = $\frac{\text{Ur Cr X Ur volume (ml)}}{\text{S Cr X Time (min.)}}$

Normal range : M= 97 to 137 mL/min (1.65 to 2.33 mL/s)

- F = 88 to 128 mL/min (14.96 to 2.18 mL/s)

Reagents:

- **R1 Reagent 1:**
- Disodium phosphate 6.4 mmol/L
- Sodium hydroxide 150 mmol/L

- **R2 Reagent 2:**
- Sodium dodecyl sulfate 0.75 mmol/L
- Picric acid 4 mmol/L
- pH 4

- **R3 Reagent 3**
- Standared Creatinine 177 µmol/L (2 mg/dl)

- A normal result of serum creatinine is:
- M= 0.7 to 1.3 mg/dL (61.9 to 114.9 µmol/L)
- F = 0.6 to 1.1 mg/dL (53 to 97.2 µmol/L).
- Women often have a lower creatinine level than men. This is because women often have less muscle mass than men. Creatinine level varies based on a person's size and muscle mass.

- Urine creatinine: M= 0.6 - 1.2 mg/dl (7 - 14 mmol/24 hours)
- F= 0.5 - 1.1 mg/dl (6 - 13 mmol/24 hours)
- Clearance is often measured as milliliters per minute (mL/min) or milliliters per second (mL/s).
- Normal values are: M= 97 to 137 mL/min (1.65 to 2.33 mL/s).
- F= 88 to 128 mL/min (1.496 to 2.18 mL/s).

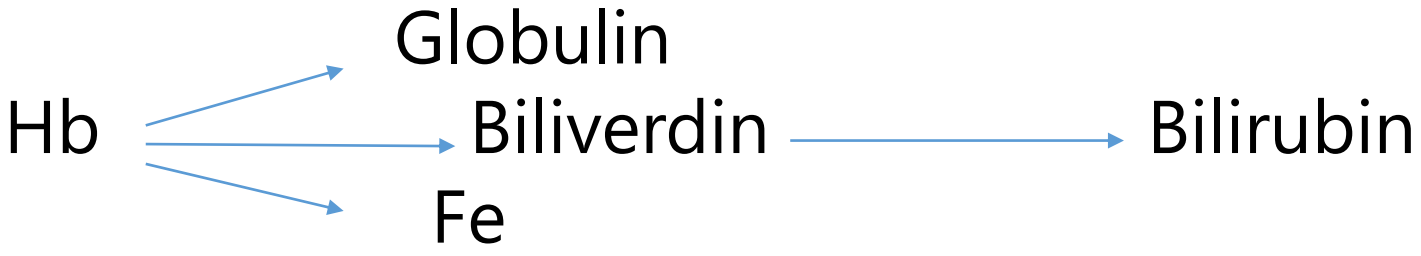
- Body surface area (BSA) (m^2) = SQRT{ [height (cm) x weight (kg)] / 3600 }

Let's try one calculation using the Mosteller formula:

1. Take your height in centimeters, e.g . 170 cm
2. Take your weight in kilograms, e.g . 60 kg
3. Multiply your height by your weight ,170X60=10200
4. Divide the result by 3600 =10200/3600 = 2.8
5. Find the square root of the result, SQRT of 2.8 = 1.67
6. If you are 170 cm tall and weigh 60 kg, the approximate calculation of your body surface area is 1.67 meters squared.

Total and Direct Bilirubin

Red cells are broken down at the end of their life in the reticulo-endothelial system mainly in the spleen. The released Hb is split into globin, which enters the general protein pool, and haem, which is converted to bilirubin after removal of iron. The iron **is** reutilized and 80% of bilirubin is metabolised daily by this process.



Other sources of bilirubin are from the breakdown of immature red cells in the bone marrow and of Hb – related compounds such as myoglobin and cytochromes.

Unconjugated bilirubin (free or indirect, that is bilirubin which has not yet been made water-soluble by conjugation with glucuronate) is bound to plasma albumin and carried to the liver.

At the hepatic cell membrane, bilirubin is removed from the albumin, then conjugated with glucuronic acid forming bilirubin diglucuronide. The reaction is catalysed by the enzyme uridyl diphosphate glucuronyl transferase (UDP).

The conjugated or direct bilirubin is water soluble. Normally, about 300 mg of bilirubin reaches the liver and is conjugated daily.

The unconjugated (indirect or free) bilirubin is water insoluble and lipid-soluble and can enter and damage brain cells.

Conjugated bilirubin is secreted into bile canaliculus and further to gall bladder for storage, concentration and excretion into small intestine.

In the intestine, bacteria deconjugate the bilirubin glucuronate and reduce bilirubin to urobilinogen.

Most of this urobilinogen is excreted in the faeces as such or as urobilin after oxidation in air.

A small portion is reabsorbed through the enterohepatic circulation to be excreted in the urine.

Therefore, normal urine contains urobilinogen but not bilirubin. However, the disappearance of urobilinogen and appearance of bilirubin (direct) in the urine is an abnormal condition that indicate the presence of obstructive jaundice.

Normally, healthy hepatic cells are capable of handling the conjugation and excretion of bilirubin.

Jaundice is due to an increase in the concentration of bilirubin in blood and is, a common sign in hepatic or biliary tract disorders

Normal range:

Serum Total bilirubin: 0.1 to 1.2 mg/dL (1.71 to 20.5 µmol/L) .

Direct bilirubin (also called conjugated): less than 0.3 mg/dL (less than 5.1 µmol/L)

The abnormal metabolism or retention of bilirubin results in jaundice which can be classified as follows:-

Types of jaundice

A- Pre-hepatic jaundice (hemolytic) (unconjugated).

1- Acute and chronic hemolytic anemia.

2- Neonatal physiological jaundice.

B- Hepatic jaundice (mainly unconjugated).

1- Conjugation failure.

2- Transport disturbances.

3- Hepatocellular damage or necrosis, viral and toxic hepatitis and cirrhosis.

4- Intrahepatic obstruction (conjugated and unconjugated).

C- Post-hepatic jaundice (obstruction of common bile duct due to stones, tumor or spasm and stricture (mainly conjugated).

Principle

Sulfanilic acid reacts with **sodium nitrite** to form **diazotized sulfanilic acid.**

Sulfanilic acid +Sodium nitrite ⟶ diazotized sulfanilic acid.

In the **presence** of **dimethyl sulfoxide**, total bilirubin reacts with diazotized sulfanilic acid to form azobilirubin.

T.bilirubin+diazotized sulfanilic A ⟶ (dimethyl sulfoxide) azobilirubin

In the **absence** of **dimethylsulfoxide**, only direct bilirubin reacts with diazotized sulfanilic acid to form azobilirubin.

Direct bilirubin+diazotized sulfanilic acid ⟶ azobilirubin

Read the absorbance of the assay azobilirubin at 550 nm using blank

Procedure: (Kit method) (BIOLABO)

Pipette into well identified test tubes :	TOTAL BILIRUBIN		DIRECT BILIRUBIN	
	Blank	Assay	Blank	Assay
Reagent R1	1 mL	1 mL		
Reagent R2			1 mL	1 mL
Distilled water	50 µL		50 µL	
Reagent R3 (Nitrite)		50 µL		50 µL
Mix				
Specimen	100 µL	100 µL	100 µL	100 µL
Mix well and start a timer when adding specimen. Read absorbances at 550 nm (530-580) against blanks TB : reading after ≥ 3 minutes at 37°C or ≥ 5 minutes at room temperature. DB : reading at **exactly** 3 minutes at 37°C or 5 minutes at room temperature.				

R1 : Sulfanilic acid +Hydrochloric acid + Dimethyl sulfoxide

R2 : Sulfanilic acid + Hydrochloric acid

Calculation:

Serum Total Bilirubin Conc.= $A_T - A_B$ X Factor

Serum Direct Bilirubin Conc.= $A_T - A_B$ X Factor

Factor= 11.4 mg/dl or 195 μmol/L

Normal level of Total bilirubin: 0.1 to 1.2 mg/dL (1.71 to 20.5 µmol/L) .

Normal level of Direct bilirubin (also called conjugated) is less than

0.3 mg/dL (less than 5.1 µmol/L)

Indirect bilirubin = Total Bilirubin – Direct bilirubin (Unconjugated)

Total and Direct Bilirubin

(Factor= 11.4 mg/dl or 195 μmol/L)

Serum Total Bilirubin Conc.= AT – AB X Factor

Serum Total Bilirubin Conc.= 0.????X 11.4 = !!!!!! mg/dl **or**

Serum Total Bilirubin Conc.= 0.????X195 = !!!!!! μmol/L

Normal level of Total bilirubin= 0.1 to 1.2 mg/dL (1.71 to 20.5 μmol/L) .

Serum Direct Bilirubin Conc = 0.????X 11.4 = !!!!!! mg/dl **or**

Serum Direct Bilirubin Conc = 0.????X195 = !!!!!! μmol/L

Normal level of Direct bilirubin(conjugated) is < 0.3 mg/dL (< 5.1 μmol/L)

Indirect bilirubin = Total Bilirubin – Direct bilirubin
(Unconjugated)

Reagents

R1 : Sulfanilic acid	30 mmol/l
Hydrochloric acid	150 mmol/l
Dimethyl sulfoxide	7 mmol/l
R2 : Sulfanilic acid	30 mmol/l
Hydrochloric acid	150 mmol/l
R3 : Sodium nitrite	20 mmol/l

ALKALINE PHOSPHATASE ACTIVITY

This is a widely distributed enzyme which releases inorganic phosphate from many organic phosphomonoesters and also pyrophosphates. The enzyme exhibits optimum activity at PH 9 – 10.

The form present in the sera of normal adults originates mainly in the liver or biliary tract and bone (osteoblasts). The respective contribution of these two forms is markedly age-dependent with a high alkaline phosphate (bone) occurring during childhood and adolescence.

Minor contribution also comes from intestine, placenta (during pregnancy) and rarely renal tissues.

Although the precise metabolic function of the enzyme is not yet understood, it appears that the enzyme is associated with calcification process in bone and lipid transport in other tissues.

Clinical significance

- Serum alkaline phosphatase is of particular interest in the investigation of two groups of conditions, hepatobiliary diseases and bone diseases associated with increased osteoblastic activity.
- The elevation tends to be more marked (more than three folds) in extrahepatic obstruction (e.g. by stone or by cancer of the head of pancreas) than intrahepatic.
- Liver diseases that principally affect parenchymal cells such as hepatitis, typically has normal or moderately elevated serum alkaline phosphatase activity, the degree of elevation is usually less than three folds and depends on the degree of biliary stasis.

- Among bone diseases the **highest level** of serum alkaline phosphatase activity is encountered in **paget's disease** (paget's disease disrupts the normal cycle of bone renewal, causing bones to become weakened and possibly deformed). **Moderate rises** are observed in **osteomalacia and rickets** (with its **activity** tends to decrease following Vit. D therapy), **primary and secondary hyperparathyroidism** with skeletal involvement, and in patients with **osteogenic bone cancer including metastases**.
- An **increase** in alkaline phosphatase activity may be observed in women in the **third trimester of pregnancy** with additional enzyme being of placental origin. Serum alkaline phosphatase should, therefore, be interpreted with caution in pregnant women.
- Normal serum level of ALP is 20-90 I.U/L (3-13) king Armstrong units/dl (K.A.U./dl) in adults.

Measurement of Serum Alkaline Phosphates Activity (ALP)

- **Principle:** (Kit method)(BIOLABO)
- The **phenol** released by enzymatic hydrolysis from **phenyl phosphate substrate** at a constant condition of time, temp. and PH is estimated colorimetrically.
- The phenol liberated is measured in the presence of amino-4-antipyrine and potassium ferricyanide where red color is formed and its absorbance is measured at 510 nm that is proportional to ALP activity.

Phenyl phosphate $\xrightarrow[\text{pH 10}]{\text{Alkaline phosphatase}}$ phenol + phosphate

- Phenol + Amino-4-antipyrine +Potassium Ferricyanide $\longrightarrow$ Red color
- The presence of sodium arsenate in the reagent stops the enzymatic reaction.

Procedure: (Kit method) (BIOLABO)

Prepare test tubes as follows	Blank	Standard	Test
Reagent 1 (substrate Buffer)	2 ml	2 ml	2 ml
Incubate for 5 minutes at 37°C			
Serum	———	———	50 µl
Reagent 2 (Standard 20 K.K.U)	———	50 µl	———
Incubate for exactly 15 minutes at 37°C			
Reagent 3: amino-4-antipyrine + sodium arsenate	0.5 ml	0.5 ml	0.5 ml
Mix well or preferably vortex.			
Reagent 4 (potassium ferricyanide)	0.5 ml	0.5 ml	0.5 ml
Serum	50 µl		
Mix, let stand for 10 minutes in the dark. Read ((A)) at 510 nm against blank at 510 nm			———

Calculation

- Alk. ph. Activity $= \frac{A_T - A_b}{A_{st} - A_b} \times n$ where n = 20 kind and king unit

Kind & King Units/100ml

- N.R. For adults is 4.5-13 kind and king unit/dl
- **ALKALINE PHOSPHATASE ACTIVITY** children is double adults value
- N.R for adults is 32-92 IU/L for children is double adults value

- **Note 1:**
- one kind and king unit is that amount of enzyme which in the given conditions liberate 1 mg of phenol in 15 minutes at 37°C and pH 10.
- **Note 2:**
- IU/L = 7.09 X (Result of kind and king units/dl)

ALKALINE PHOSPHATASE ACTIVITY

Alk. ph. Activity = $\frac{A_T - A_b}{A_{st} - A_b} \times n$ where n = 20 kind and king unit
Kind & King Units/100ml

Alk. ph. Activity = $\frac{0.???}{0.???}$ x 20 = !!!! kind and king unit/dl

N.R. For adults is 4.5-13 kind and king unit/dl. For children is double adults value

Alk. ph. Activity in IU/L = 7.09 X (Result of kind and king units/dl)

= 7.09 X !!!!

N.R for adults is 32-92 IU/L . For children is double adults value.

- **Note :**
- one kind and king unit is that amount of enzyme which in the given conditions liberate 1 mg of phenol in 15 minutes at 37°C and pH 10.

Reagents (Kit method) (BIOLABO)

- **R1**: **Substrate-Buffer**
- Disodium phenyl phosphate 5 mmol/L
- Carbonate-Bicarbonate buffer pH 10 50 mmol/L

- **R2: Standard**
- Phenol corresponding to 20U king and kind

- **R3: Bloking reagent**
- 4-Amino antipyrine 60mmol/L
- Sodium arsenate 240 mmol/L
- **R4: Dye reagent**
- Potassium Ferricyanide 150 mmol/L

The Liver

Liver function Tests

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Lectures in Clinical Chemistry

Introduction

- Liver
  - Largest internal organ; functionally complex
  - Plays critical role in:
    - Metabolism
    - Digestion
    - Detoxification
    - Elimination of substances from body
  - Unique in resilience, ability to regenerate cells destroyed by short-term injury or disease

Anatomy

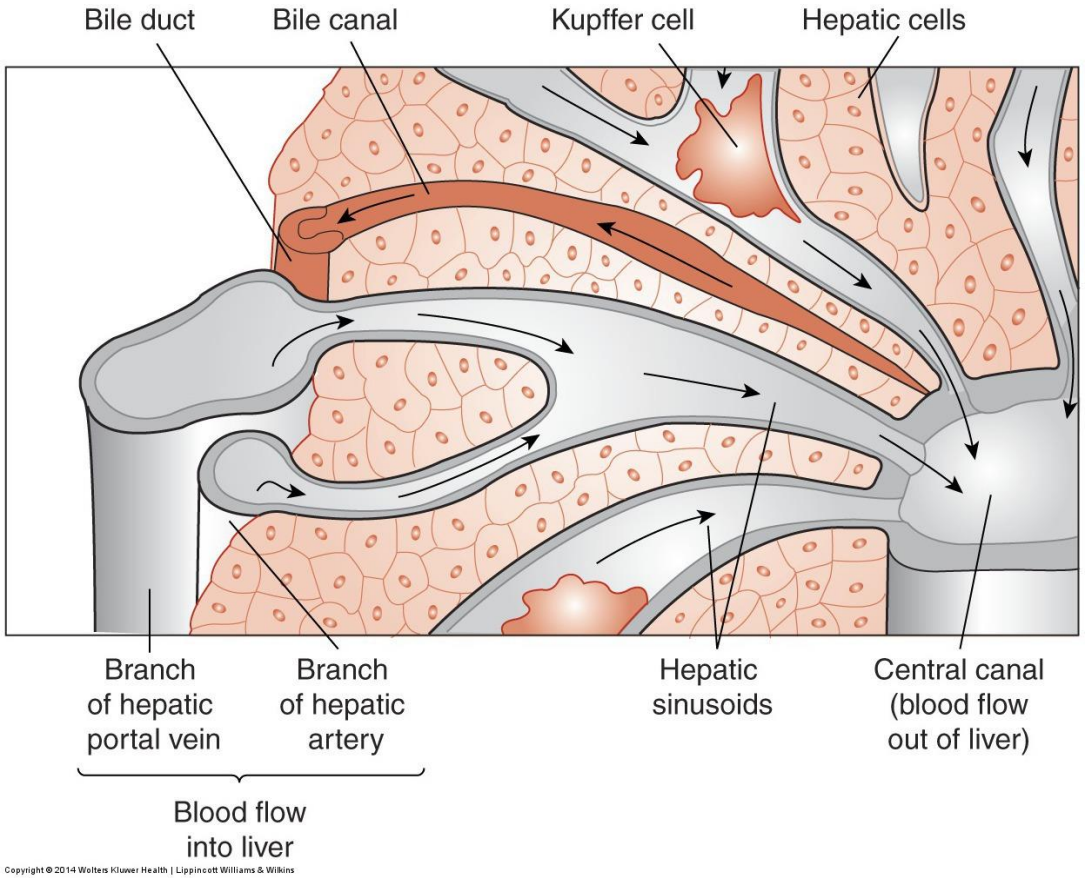
- Gross Anatomy
  - Liver weighs 1.2–1.5 kg in healthy adult
  - Located beneath & is attached to diaphragm, protected by rib cage, held in place by ligamentous attachments
  - Divided unequally into 2 lobes by falciform ligament
  - Extremely vascular; receives blood from 2 sources:
    - Hepatic artery (supplies 25%)
    - Portal vein (supplies 75%)
  - **Bile canaliculi:** small spaces between hepatocytes that form intrahepatic ducts where excretory products of cell can drain

Anatomy (cont'd)

- Microscopic Anatomy
  - Lobules
    - Microscopic units that divide liver
    - Responsible for all metabolic & excretory functions
    - 6-sided structures with centrally located vein & portal triads
    - Portal triad contains hepatic artery, portal vein, bile duct.
  - Two major cell types in liver
    - **Hepatocytes:** large cells radiating outward from central vein
    - **Kupffer cells:** macrophages lining sinusoids; act as phagocytes, engulfing bacteria, debris, toxins

Anatomy (cont'd)

- Microscopic anatomy of the liver



Biochemical Functions

- Excretory and Secretory
  - Liver is only organ with capacity to rid body of heme waste.
  - Bile & Bile salts.
    - Made up of bile acids or salts, bile pigments, cholesterol
    - Body produces 3 L of bile per day & excretes 1 L.
  - Bilirubin
    - Principal pigment in bile, derived from breakdown of red blood cells
    - 200–300 mg produced per day
    - Most is eliminated in feces, some in urine.

Biochemical Functions (cont'd)

- Metabolism
  - Carbohydrate synthesis
    - Liver maintains stable glucose concentrations by storing it as glycogen & degrading glycogen when needed by body.
  - Lipid synthesis
    - Liver breaks down fatty acids to form triglycerides, phospholipids, or cholesterol.
  - Protein synthesis
- Detoxification and Drug Metabolism
  - Liver prevents toxic or harmful substances from reaching systemic circulation by binding or chemical modification.

Major functions of the liver

Liver Function Alterations During Disease

- Jaundice
  - Yellow discoloration of skin, eyes, & mucous membranes
  - Results from retention of bilirubin or other substances
  - Classified based on site of disorder:
    - **Prehepatic:** problem occurs before liver
    - **Hepatic:** problem occurs in liver
    - **Posthepatic:** problem occurs after liver

Liver Function Alterations During Disease (cont'd)

- Cirrhosis
  - Condition in which scar tissue replaces healthy liver tissue
  - Scar tissue blocks blood flow & prevents proper functioning.
  - Commonly caused by chronic alcoholism & hepatitis C infection
    - Also: chronic Hep B and D; autoimmune disorders, and some inherited causes.
- Tumors
  - 90–95% of hepatic malignancies are metastatic (secondary), not originating in liver cells (not primary).
  - **Benign:** hepatocellular adenoma, hemangiomas
  - **Malignant:** hepatocellular carcinoma, hepatocarcinoma, hepatoma

Liver Function Alterations During Disease (cont'd)

- Drug- and Alcohol-Related Disorders
  - Drug-induced liver disease accounts for 1/3 to 1/2 of all reported cases of acute liver failure in western countries.
  - Most common mechanism of injury is adverse immune response to drug directed against liver.
  - Ethanol (alcohol) is most significant cause of hepatic toxicity.
  - Three stages of liver injury due to excessive alcohol consumption
    - 1. **Alcoholic fatty liver:** mild; recovery with removal of drug
    - 2. **Alcoholic hepatitis:** evidence of liver damage
    - 3. **Alcoholic cirrhosis:** most severe; poor prognosis

Liver Function tests

- ***Bilirubin*** ;

- Bilirubin in blood & urine are used to assess hepatic transport

  80% of bilirubin formed each day arises from red cells. 20% comes from red cell precursor (ineffective erythropoiesis)& from haem proteins as myoglobin ,cytochromes and peroxidase .

  Iron is removed and the porphyrin ring is opened to form bilirubin.

Structure of bilirubin

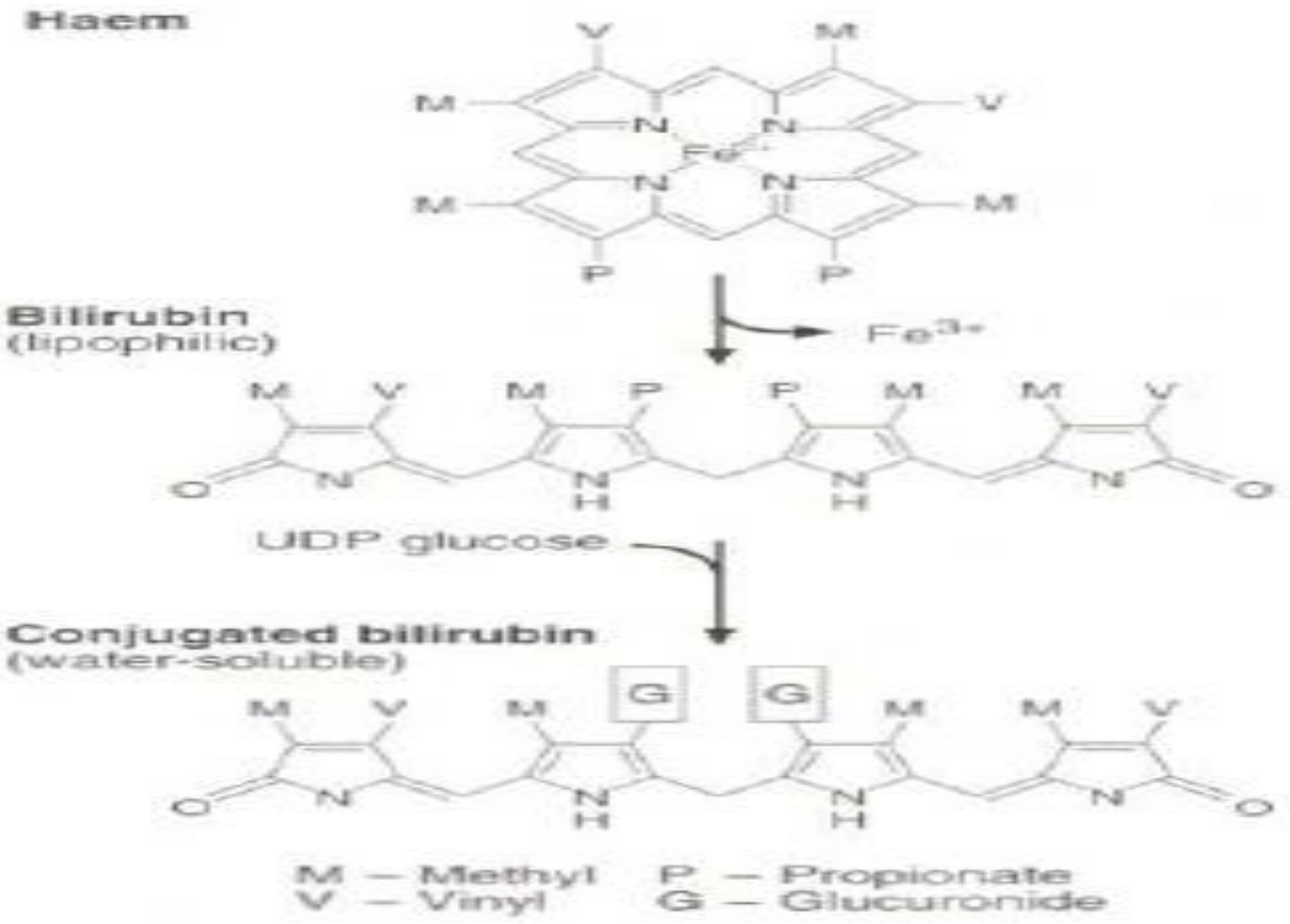
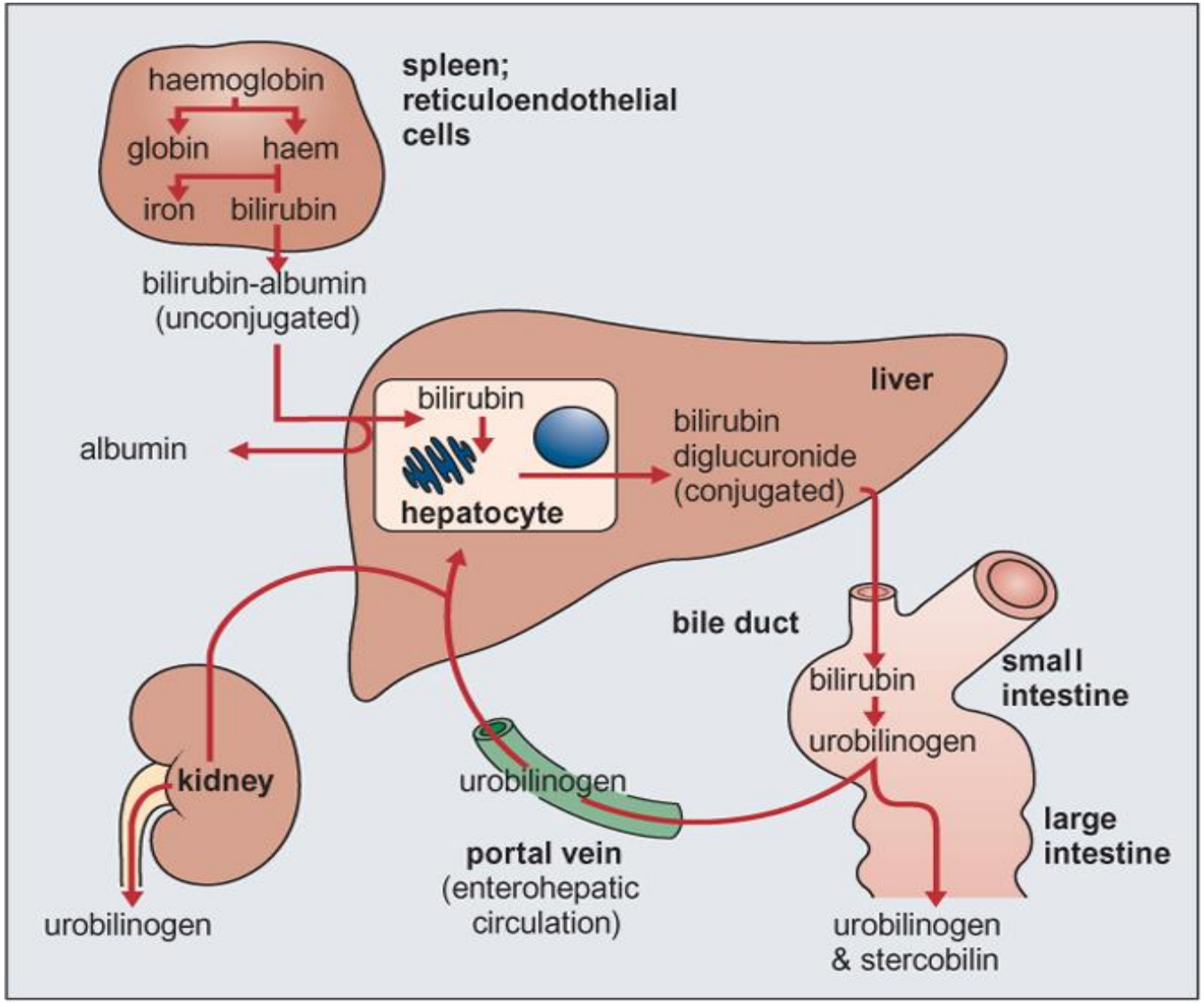


Fig. 2 **Structure of bilirubin and conjugated bilirubin.**

Transport in plasma and hepatic uptake

- Bilirubin is insoluble in water and carried with albumin in blood
- **Conjugation of bilirubin and secretion into bile : UDP-glucuronyltransferase ,conjugates bilirubin with glucuronic acid.which is water soluble , degraded in colon by bacteria to urobilinogen then oxidized to brown uroblins & stercobilins.**

Excretion of bilirubin



Assessment of Liver Function/Liver Function Tests (cont'd)

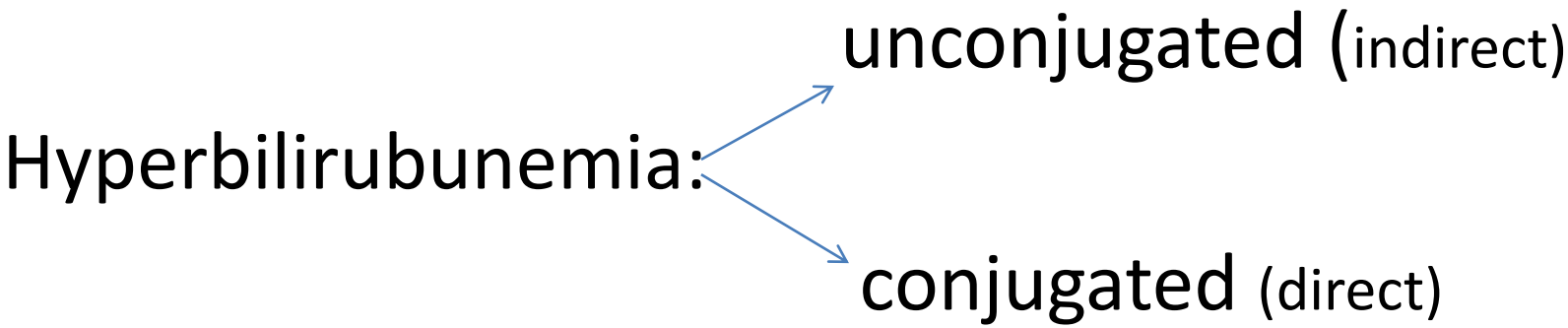
- Bilirubin
  - Specimen Collection and Storage
    - May be performed on serum (preferred) or plasma
    - Fasting sample is preferred.
    - Is sensitive to light & should be protected
    - Avoid hemolysis of sample.
  - Methods
    - Most commonly used are Jendrassik-Grof or Malloy Evelyn.

Assessment of Liver Function/Liver Function Tests (cont'd)

➢ Urobilinogen in Urine and Feces

- Urobilinogin is a colorless end product of bilirubin metabolism that is oxidized by intestinal bacteria to the brown pigment urobilin and stercobilin
- In the normal individual, part of the urobilinogen is excreted in the **feces**, and the remainder is reabsorbed into the portal blood and returned to the **liver.** A small portion that is not taken up by the hepatocytes is excreted by the **kidney** as urobilinogen
- Increased levels of urinary urobilinogen are found in hemolytic disease and in defective liver-cell function, such as hepatitis
- **Absence of urobilinogen** from the urine and stool is most often seen with complete **biliary obstruction.** Fecal urobilinogen is also decreased in partial biliary obstruction and in hepatocellular disease

Types of hyperbilirubinemia



Jaundice

- Although all cases of jaundice result from hyperbilirubinemia, not all are caused by hepatic dysfunction.
- hyperbilirubinemia may also result from erythrocyte destruction, or hemolysis in patients with normal liver function
- Hypercarotenemia (excessive ingestion of vitamin A) may produce skin discoloration indistinguishable from that of hyperbilirubinemia. In hypercarotenemia, the sclerae are usually not discolored.

Jaundice

Hyperbilirubinemia may be caused by:
-Increased production
-Impaired metabolism
-decreased excretion
- Combination of these

Jaundice is due to hyperbilirubinaemia ;

- Prehepatic
- Hepatocellular
- Cholestatic (post hepatic)

Causes of jaundice

Major causes of jaundice	
Pre-hepatic	**Post-hepatic**
haemolysis ineffective erythropoiesis	gallstones biliary stricture carcinoma of pancreas or biliary tree cholangitis
Hepatic	
pre-microsomal drugs, e.g. rifampicin, which interfere with bilirubin uptake microsomal prematurity hepatitis, e.g. viral or drug-induced Gilbert's syndrome Crigler–Najjar syndrome	post-microsomal impaired excretion hepatitis drugs, e.g. methyltestosterone, rifampicin Dubin–Johnson syndrome intrahepatic obstruction hepatitis cirrhosis infiltrations, e.g. lymphoma, amyloid biliary atresia tumours extrahepatic sepsis

Marshall & Bangert: Clinical Chemistry, 6th Edition.

Prehepatic hyperbilirubinaemia;

Increased [unconjugated bil] ,due to;

- Hemolytic anemia
- Ineffective erythropoiesis e.g pernicious aenemia
- bleeding

Hepatocellular hyperbilirubinaemia

- Drugs or toxins
- Cirrhosis
- Low activity of UDP glucouronyltransferase in congenital deficiency (Gilberts syndrome & Crigler - Najjar syndrome)
- Pre mature infants
- Dubin Jonson syndrome (benign)

Inherited disorders of bilirubin metabolism

Inherited disorders of bilirubin metabolism		
Syndrome	**Defect**	**Clinical features**
Gilbert's	decreased conjugation of bilirubin and decreased uptake in some cases (autosomal dominant)	mild, fluctuant unconjugated hyperbilirubinaemia that increases on fasting normal biopsy normal lifespan
Crigler–Najjar	Type 1 (autosomal recessive) absence of conjugating enzyme	severe unconjugated hyperbilirubinaemia early death due to kernicterus partial response to phototherapy, none to phenobarbital
	Type 2 (autosomal dominant with variable penetrance) partial defect of conjugating enzyme	severe unconjugated hyperbilirubinaemia but good response to phenobarbital and phototherapy often survive to adulthood
Dubin–Johnson	decreased hepatic excretion of bilirubin (autosomal recessive)	mild, fluctuant conjugated hyperbilirubinaemia hepatic pigment disposition (melanin) increased coproporphyrin I/III ratio in urine bilirubinuria normal lifespan
Rotor	precise defect unknown (autosomal recessive)	similar to Dubin–Johnson but no hepatic pigmentation

Cholestatic hyperbilirubinaemia

There is conj.hyperbilirubinaemia with bilirubinuria;**intrahepatic causes** ;

- Infectious
- Carcinoma
- Cirrhosis
- Drugs

Extra hepatic causes;

- Gallstone
- Carcinoma of the head of the pancreas
- Carcinoma of the head of the biliary tree

Assessment of Liver Function/Liver Function Tests (cont'd)

- Enzymes
  - Are released into circulation after an injury that results in cytolysis or necrosis
  - Used to differentiate hepatocellular from obstructive liver disease

Assessment of Liver Function/Liver Function Tests (cont'd)

- Enzymes
  - Aminotransferases
    - Aspartate aminotransferase (AST)
    - Alanine aminotransferase (ALT)
  - Phosphatases
    - Alkaline phosphatase (ALP)
    - Gamma-glutamyltransferase (GGT)
    - Lactate dehydrogenase (LDH)

Enzyme Tests in Liver Disease

- Any injury to the liver that results in cytolysis and **necrosis** causes the liberation of various enzymes.

- The most common enzymes assayed in hepatobiliary disease include alkaline phosphatase (ALP), and the aminotransferases (AST & ALT).

- Used less often are γ-glutamyltransferase (GGT), lactate dehydrogenase (LD) and its isoenzymes, 5'-nucleotidase, ornithine carbamoyltransferase (OCT).

Assessment of Liver Function/Liver Function Tests (cont'd)

- Tests Measuring Hepatic Synthetic Ability
  - Serum albumin
    - Decreased level may be caused by decreased liver protein synthesis.
  - Prothrombin time
    - Commonly increased in liver disease
- Tests Measuring Nitrogen Metabolism
  - Plasma ammonia level
    - Increased level occurs with liver failure.

Plasma proteins of diagnostic value in liver diseases

Plasma proteins of diagnostic value in liver disease		
Protein	**Condition**	**Change in concentration**
albumin	chronic liver disease	↓
γ-globulins	cirrhosis, especially autoimmune	↑
α_1-antitrypsin	cirrhosis due to α_1-antitrypsin deficiency	↓
caeruloplasmin	Wilson's disease	↓
α-fetoprotein	primary hepatocellular carcinoma	greatly ↑
transferrin	haemochromatosis	normal but 100% saturated with iron
ferritin	haemochromatosis	greatly ↑

Biochemistry of specific liver diseases

Acute liver disease;

- Acute hepatitis, caused by:
  - viral infection:hepatitis viruses A,B,C,D, & E.
  - toxins e.g: alcohol, paracetamol, CCl_4, and fungal toxins.

- Chronic hepatitis
  - hepatic inflammation persisting more than 6 months:
  - autoimmune hepatitis
  - chronic infection with hepatitis B & C
  - alcohol

Specific Disorders

- Infiltration of the liver

Carcinoma , amyloidosis, tuberculosis ,sarcoidosis, abscesses. These diseases leads to biliary obstruction.

- Cirrhosis of the liver

Alcoholism , viral hepatitis , Wilsons disease, cystic fibrosis, haemochromatosis , galactosaemia.

Drug- and Alcohol-Related Disorders

- Many drugs and chemicals are toxic to the liver. This toxicity may take the form of overwhelming hepatic necrosis, leading to coma and death, or it may be subclinical and pass entirely unnoticed

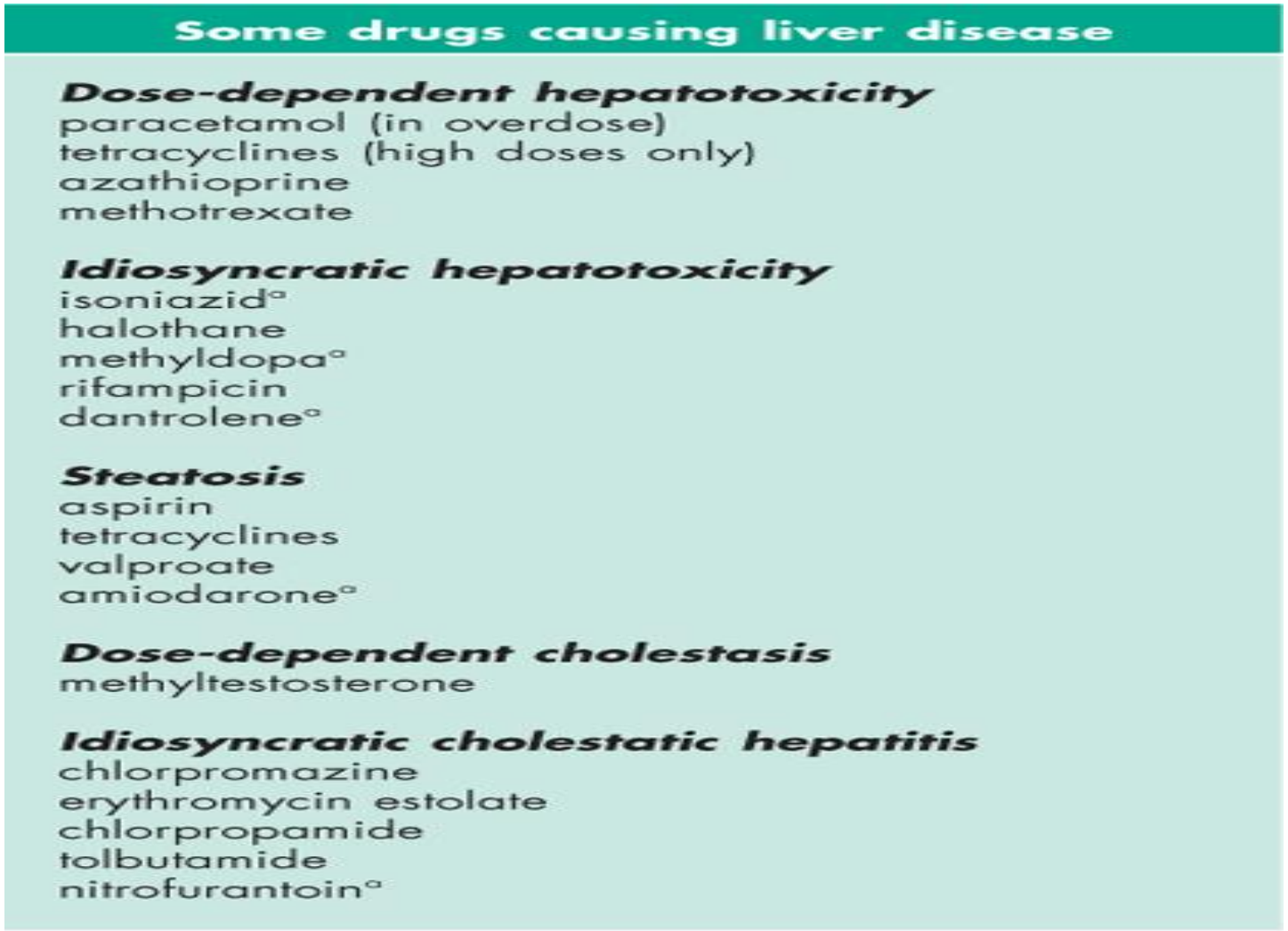
- In small amounts, **alcohol** may cause mild, inapparent injury. Heavier consumption leads to more serious damage, and prolonged, heavy use may lead to cirrhosis (exact amount is unknown)

- Certain **drugs**, including tranquilizers (phenothiazines), certain antibiotics, antineoplastic agents, and anti-inflammatory drugs, may cause liver injury

- Usually this is mild and manifested only by elevation of liver function tests, which return to normal when the drug is discontinued. This may lead to massive hepatic failure or cirrhosis

- The most common drug associated with serious hepatic injury is **acetaminophen**. When taken in massive overdose, it produces fatal hepatic necrosis unless rapid treatment is initiated

Some drugs causing liver dsease



Cirrhosis

- Cirrhosis is a serious disorder and one of the ten leading causes of death in western countries. It causes many complications:
  - **Portal hypertension** results when blood flow through the portal vein is obstructed by the cirrhotic liver.
  - The synthetic ability of the liver is reduced, causing **hypoalbuminemia** and deficiency of the clotting factors, which may lead to hemorrhage
  - **Ascitic fluid** may accumulate in the abdomen

Tumors of the Liver

- On a worldwide basis, primary malignant tumors of the liver, known as hepatocellular carcinoma are an important cause of cancer mortality
- Usually , these tumors are relatively uncommon. Most cases of hepatocellular carcinoma can be related to previous infection with a hepatitis virus.
- Liver is frequently involved secondarily by tumors arising in other organs. Metastatic tumors to the liver from primary sites, such as the lung, pancreas, gastrointestinal tract, or ovary, are common. Benign tumors of the liver are relatively uncommon
- Whether primary or secondary any malignant tumor in the liver is a serious finding with a poor prognosis
- The only hope For cure relies on surgical resection, which is usually impossible. Patients with malignancies of the liver usually have a survival measured in months

Abnormalities of simple liver function tests

Test	Condition				
	Acute hepatitis	Chronic hepatitis	Cirrhosis	Cholestasis	Malignancy and infiltrations
Bilirubin	N to ↑↑	N to ↑	N to ↑	↑ to ↑↑↑	N
Aminotransferases	↑↑↑	↑	N to ↑	N to ↑	N to ↑
Alkaline phosphatase	N to ↑	N[b]	N to ↑↑	↑↑↑	↑↑
Albumin	N	N to ↓	N to ↓	N	N to ↓
γ-Globulins	N	↑	↑	N	N
Prothrombin time	N to ↑[a]	N to ↑	N to ↑[a]	N to ↑[c]	N

Assessment of Liver Function/Liver Function Tests (cont'd)

- Hepatitis
  - Injury to liver characterized by inflammation in liver tissue
  - **Causes:** viral, bacterial, & parasitic infections, radiation, drugs, chemicals, autoimmune diseases and toxins
  - **Symptoms:** jaundice, dark urine, fatigue, nausea, vomiting, abdominal pain
  - **Hepatitis A**
    - Most common form of viral hepatitis worldwide
    - Caused by infection with the hepatitis A virus (HAV) via contaminated or improperly handled food (oral–fecal route)

Assessment of Liver Function/Liver Function Tests (cont'd)

- Hepatitis
  - **Hepatitis B**
    - Can cause both acute & chronic hepatitis
    - Caused by infection with hepatitis B virus (HBV) via parenteral, perinatal, & sexual transmission
    - Serologic markers of HBV infection: HBcAg, HBsAg, HBeAg
  - **Hepatitis C**
    - Caused by infection with hepatitis C virus (HCV) via parenteral transmission (primarily by blood transfusion)

Assessment of Liver Function/Liver Function Tests (cont'd)

- Hepatitis
  - **Hepatitis D**
    - A unique subvirus satellite virus infection
    - Requires HbsAg of HBV for replication; can only occur in patients who already have hepatitis B
  - **Hepatitis E**
    - Caused by infection with hepatitis E virus (HEV): a nonenveloped RNA virus that is only 27–34 nm in diameter
    - Transmitted primarily by fecal–oral route
    - Characterized by water-borne epidemics in developing countries

Plasma proteins and enzymes

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Functions of plasma proteins

Function	Example
transport	thyroxine-binding globulin (thyroid hormones) apolipoproteins (cholesterol, triglyceride) transferrin (iron)
humoral immunity	immunoglobulins
maintenance of oncotic pressure	all proteins, particularly albumin
enzymes	renin coagulation factors complement proteins
protease inhibitors	α_1-antitrypsin (acts on proteases)
buffering	all proteins

Causes of changes in total plasma protein concentration

Increase		Decrease	
hypergammaglobulinaemia paraproteinaemia	↑ protein synthesis	malnutrition and malabsorption liver disease humoral immunodeficiency	↓ protein synthesis
artefactual	haemoconcentration due to stasis of blood during venepuncture	over-hydration increased capillary permeability	↑ volume of distribution
dehydration	↓ volume of distribution	protein-losing states catabolic states	↑ excretion/catabolism

Principal plasma proteins

Class	Protein	Approximate mean serum concentration (g/L)
	prealbumin	0.25
	albumin	40
α_1-globulin	α_1-antitrypsin	2.9
	α_1-acid glycoprotein	1.0
α_2-globulin	haptoglobins	2.0
	α_2-macroglobulin	2.6
	caeruloplasmin	0.35
β-globulin	transferrin	3.0
	low density lipoprotein	1.0
	complement components (C3)	1.0
γ-globulins	IgG	14.0
	IgA	3.5
	IgM	1.5
	IgD	0.03
	IgE	trace

Marshall & Bangert: Clinical Chemistry, 6th Edition.

Albumin

- **Albumin is the protein present in highest concentration in the serum that is synthesized in the liver.**
- **Because of its high concentration in blood, albumin is responsible for nearly 80% of oncotic pressure**
- **Albumin binds bilirubin, salicylic acid, fatty acids, calcium and magnesium ions, cortisol, and some drugs. This characteristic is also exhibited with certain dyes, providing a method for the quantitation of albumin.**

Causes of hypoalbuminaemia

Decreased synthesis

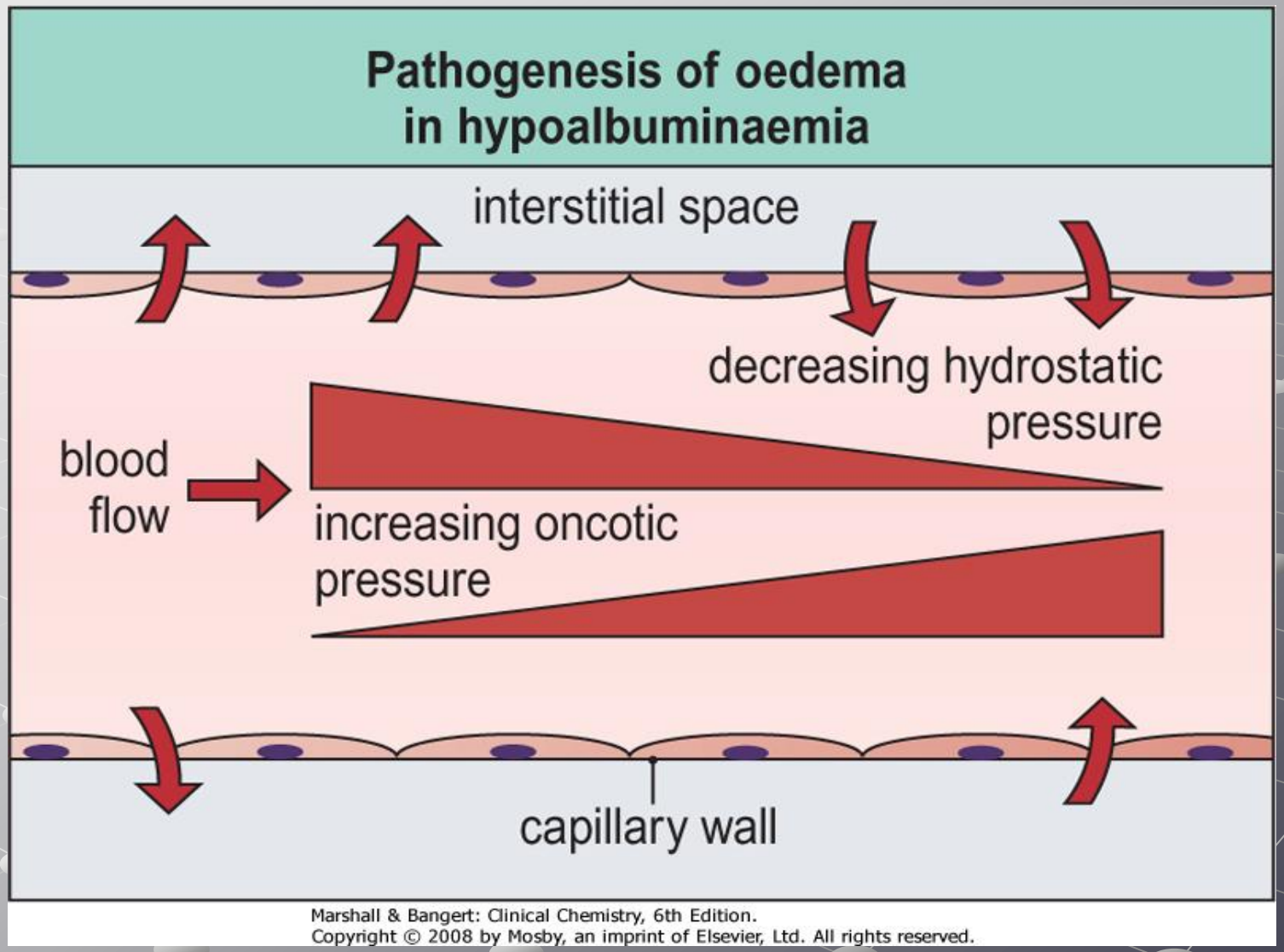
malnutrition
malabsorption
liver disease

Increased volume of distribution

over-hydration
increased capillary permeability
- septicaemia
- hypoxia

Increased excretion/degradation

nephrotic syndrome
protein-losing enteropathies
burns
haemorrhage
catabolic states
- severe sepsis
- fever
- trauma
- malignant disease



Albumin

- Increased serum albumin levels are seen in dehydration.
- Administering fluids to treat the dehydration will decrease serum albumin levels back to normal.

- When more information about proteins is needed, an electrophoretic pattern is obtained, and the albumin is calculated as a percentage of the total protein (usually, approximately 60%)

- At birth, the reference value for serum albumin averages 39 g/L. The concentration falls to 28.4 g/L at about 9 months and then begins to increase slowly until adult values of 35-55 g/L are reached.

α1-Antitrypsin

- It is a naturally occurring inhibitor of proteases
- It is an acute phase protein
- Its conc. increases in acute inflammatory states

- A deficiency of α1-antitrypsin is associated with severe, degenerative, emphysematous pulmonary disease due to proteolytic activity of proteases from leukocytes in the lung during periods of inflammation

- Juvenile hepatic cirrhosis is also a correlative disease in α1-antitrypsin deficiency. The protein is synthesized but not released from the hepatocyte.

- increased levels of α1-antitrypsin are seen in inflammatory reactions, pregnancy, and contraceptive use.

Haptoglobin

- It is an acute phase protein
- The function of haptoglobin is to bind free hemoglobin released into plasma during intravascular hemolysis
- The reticuloendothelial cells remove the haptoglobin-hemoglobin complex from circulation within minutes of its formation, causing a fall in haptoglobin. So a low plasma haptoglobin indicate intravascular hemolysis.
- haptoglobin prevents the loss of hemoglobin and its constituent iron into the urine.
- Serum concentration is increased in inflammatory conditions, and in nephrotic syndrome

α_2- Macroglobulin

- Its plasma conc. is increased in nephrotic syndrome
- It is also an inhibitor of proteases (like α1-antitrypsin)

Caeruloplasmin

- it is an acute phase protein
- copper containing protein
- it functions as a scavenger
- in Wilson’s disease: its synthesis and blood conc. is greatly reduced
- in pregnancy, its conc. is increased (an estrogen-related effect)

Transferrin

- is the major iron-trasporting protein in plasma
- its measurement (together with ferritin) is used as a test for the assessment of body iron store
- its measurement is also used as an index of nutritional state

Immunoglobulins

Characteristics of the immunoglobulins

Class	Heavy chain	Mean plasma concentration (g/L)	Molecular weight (kDa)	Function
IgG	γ	14.0	146	the major antibody of secondary immune responses
IgA	α	3.5	160	secreted as a dimer (molecular weight 385 kDa) the major antibody in seromucous secretions, e.g. saliva, bronchial mucus
IgM	μ	1.5	970	a pentamer, confined to the vascular spaces the major antibody of the primary immune response
IgD	δ	0.03	184	present on the surface of B-lymphocytes, involved in antigen recognition
IgE	ε	trace	188	present on surface of mast cells and basophils probable role in immunity to helminths and associated with immediate hypersensitivity reactions

Hypogammaglobulinemia

- inherited disorder

  *Bruton's disease: is a complete absence of immunoglobulins

- acquired hypogammaglobulinemia (might be a complication of cytotoxic drugs during malignancies :

  - *chronic lymphatic leukemia
  - *multiple myeloma
  - *Hodgkin's disease

- measurement of specific class of immunoglobulin is essential for the diagnosis of the type of hypogammaglobulinemia

Hyperimmunoglobulinemia

- is common in autoimmune disease:

  *rheumatoid disease

  *Systemic lupus erythematosus (SLE)

  *some liver autoimmune disease

- measurement of specific auto-antibodies is of great value in diagnosis of autoimmune diseases

Cytokines

- are low molecular weight peptides secreted by cells involved in inflammatory and immunity processes

- four classes of cytokines:

- *interleukines (IL): regulator of inflammation
- *interferon (IF): natural antiviral
- *colony-stimulating factors (CF)
- *tumor necrosis factors (TNF)

- they can be measured in serum in:

-the early diagnosis of sepsis and graft reaction

Plasma enzymes

they are either :

*actively secreted into blood e.g. :

- complement factors
- renin
- coagulation factors

*or primarily intracellular, being released into the blood in case of cellular damage

Plasma enzymes..

- in addition to tissue damage which cause increase in enzyme level in blood, other causes exist that may increase in their level, include:

*Increased cell turnover

*neoplasia

*enzyme induction

*obstruction to secretion

*decreased clearance

- usually enzyme activity is measured in plasma

Alkaline Phosphatase (ALP)

- **ALP activity is present on cell surfaces in most human tissue.**
- **The highest concentrations are found in the intestine, liver, bone, placenta, and kidney**
- **activity in bone is confined to the osteoblasts, those cells involved in production of bone matrix**

Causes of an increased plasma alkaline phosphatase activity

Physiological

pregnancy (last trimester)
childhood

Pathological

often >5 × ULN
- Paget's disease of bone
- osteomalacia, rickets
- cholestasis (intra- and extrahepatic)
- cirrhosis

usually <5 × ULN
- bone tumours (primary and secondary)
- renal bone disease
- primary hyperparathyroidism with bone involvement
- healing fractures
- osteomyelitis
- hepatic space-occupying lesions (tumour, abscess)
- infiltrative hepatic disease
- hepatitis
- inflammatory bowel disease

Aminotransferases

- **Two aminotransferases are used in diagnosis and management :**

  - ***AST**
  - ***ALT**

Causes of an increased plasma aspartate aminotransferase activity

Often >10 × ULN

acute hepatitis and liver necrosis
major crush injuries
severe tissue hypoxaemia
(levels may sometimes exceed 100 × ULN in these conditions)

5–10 × ULN

myocardial infarction
following surgery or trauma
skeletal muscle disease
cholestasis
chronic hepatitis

Usually <5 × ULN

physiological (neonates)
other liver diseases
pancreatitis
haemolysis (*in vivo* and *in vitro*)

γ-Glutamyltransferase (GGT)

- an enzyme involved in the transfer of the γ-glutamyl residue from - γ-glutamyl peptides to amino acids, H_2O, and other small peptides

- In most biologic systems, glutathione serves as the γ-glutamyl donor

- GGT activity is found primarily in tissue of the kidney brain, prostate, pancreas, and liver

- Clinical applications of assay are confined mainly to evaluation of liver and biliary system disorders.

Diagnostic Significance

- GGT is elevated in virtually all **hepatobiliary disorders**, making it one of the most sensitive of enzyme assays in these conditions. Higher elevations are generally observed in biliary tract obstruction.

- GGT levels will be increased in patients receiving **enzyme-inducing drugs** such as warfarin, phenobarbital, and phenytoin.

- Elevated GGT levels may indicate **alcoholism**, particularly chronic alcoholism (2 to 3 times ULN)

Causes of an increased plasma γ-glutamyl transferase activity

Often >10 × ULN

cholestasis
alcoholic liver disease

5–10 × ULN

hepatitis (acute and chronic)
cirrhosis (without cholestasis)
other liver diseases
pancreatitis

Usually <5 × ULN

excessive alcohol ingestion
enzyme-inducing drugs
congestive cardiac failure

LD Diagnostic Significance

- Increased levels are found in cardiac, hepatic, skeletal muscle, and renal diseases, as well as in several hematologic and neoplastic disorders
- The highest levels of total LD are seen in pernicious anemia and hemolytic disorders.
- Liver disorders, such as viral hepatitis and cirrhosis (2-3 times of normal)
- Measurement of LD activity is useful in following response to treatment in patients with tumors because there is a good correlation between its activity and tumor bulk

Diagnostic Significance of LD enzyme

- The enzyme can be separated into five major fractions, each comprising four subunits.
- Two different polypeptide chains, designated H (heart) and M (muscle), combine in five arrangements to yield the five major isoenzyme fractions
- In the sera of healthy individuals, the major isoenzyme fraction is LD-2, followed by LD-1, LD-3, LD-4, and LD-5
- Cardiac tissue and red blood cells contain a higher concentration of LD-1. Therefore, in conditions involving cardiac necrosis (MI) and intravascular hemolysis, the serum levels of LD-1 will increase to a point known as the LD flipped pattern (LD-1 > LD-2) suggestive of MI

Diagnostic Significance of LD enzyme

- Elevations of LD-3 occur most frequently with pulmonary involvement and various carcinomas

- The LD-4 and LD-5 isoenzymes are found primarily in liver and skeletal muscle tissue, LD-5 levels have greatest clinical significance in the detection of hepatic disorders, particularly intrahepatic disorders. Disorders of skeletal muscle will reveal elevated LD-5 levels, as depicted in the muscular dystrophies.

Creatine kinase

Causes of an increased plasma creatine kinase activity

Often >10 × ULN
polymyositis
rhabdomyolysis (e.g. trauma, malignant hyperpyrexia)
Duchenne muscular dystrophy
myocardial infarction

5–10 × ULN
following surgery
skeletal muscle trauma
severe exercise
grand mal convulsions
myositis
carriers of Duchenne muscular dystrophy

Usually <5 × ULN
physiological (Afro-Caribbeans)
hypothyroidism
drug (statin) treatment

Amylase

- found in:

  *salivary gland

  *and in pancreas

- plasma amylase activity increase in pancreatitis

- it is used in the diagnosis of patients with acute abdomen

The gonads

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Androgens and testicular function

- testis:

-synthesis of male sex hormones (androgens)

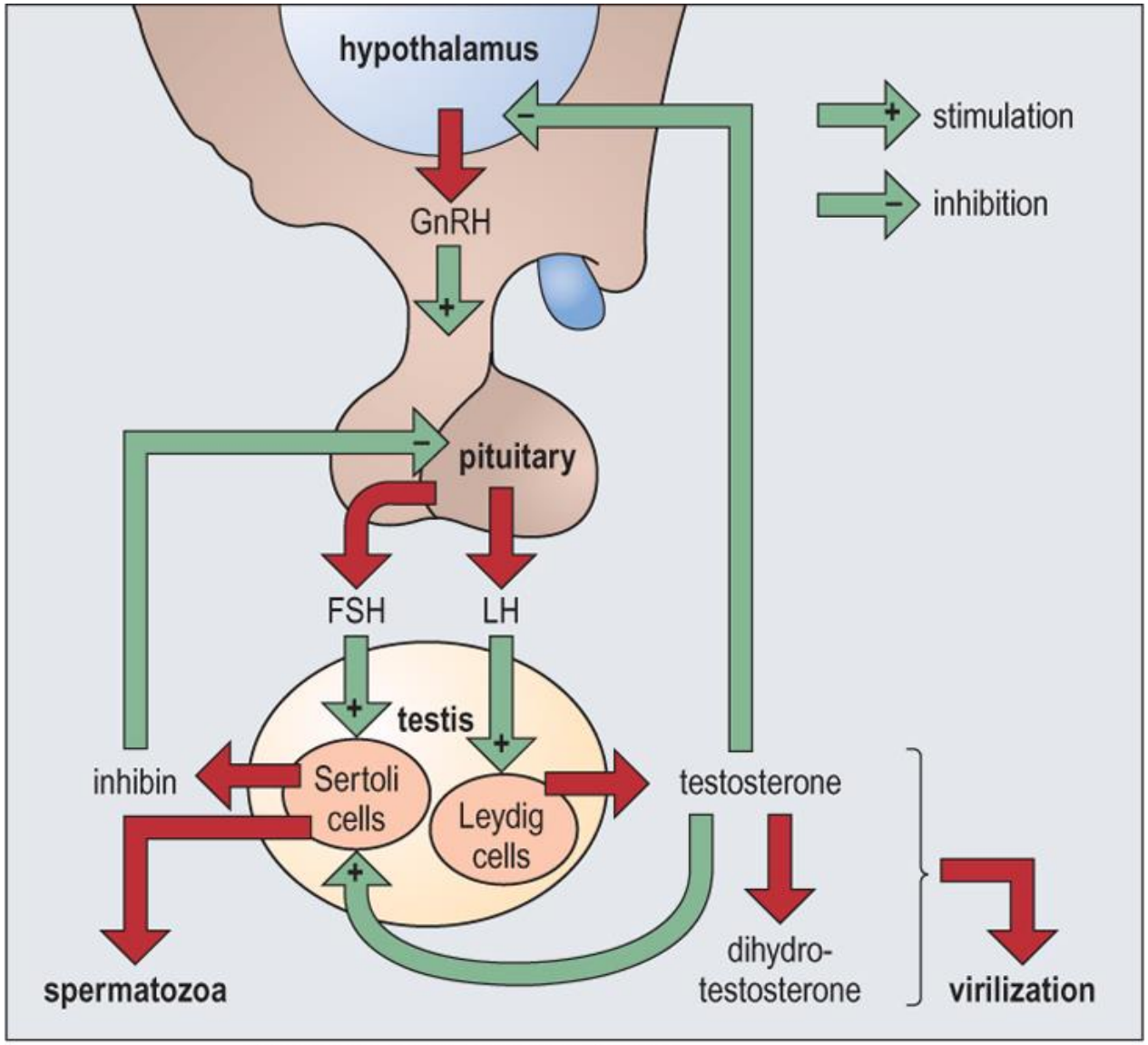
-production of spermatozoa

- testosterone is the major androgen (potency+amount)

- androstendione and DHEA: both are weak androgens

Androgens… cont..

- testosterone is a powerful anabolic hormone
- secreted from Leydig cells under the control of LH
- spermatogenesis: in Sertoli cells (dependent on FSH)
- Sertoli cell secretes inhibin: inhibit FSH
- testosterone in circulation is 97% bound to SHBG and to a less extent to albumin



Andr… cont..

- the biological activity of T is mainly to **DHT**: a metabolite of testosterone in tissues by the enzyme **5α-reductase**
- deficiency of this enz cause incomplete musculinization
- defect in T and/or DHT receptor may cause: gynecomastia or pseudohermaphroditism
- T in females: from ovaries + metabolism of adrenal androgen
- assays are available for measurement of:

  T , DHT , androstendione , DHEA , and SHBG

Estrogen and ovarian function

- 17β estradiol(E_2) is the major estrogen of the ovary

 * ovaries secretes also : estrone

 *estrogens are also secreted from placenta and corpus luteum (massive increase of estriol during pregnancy)

- estrogens responsible for secondary characteristics in females

 -stimulate growth of ovarian follicles

 -proliferation of uterine endometrium

 -affect cervical mucus and vaginal epithelium

 -functions related to reproduction

Estrogens… cont..

- estrogens are low before puberty
- increase after puberty
- estrogens fall after menopause (estrogen from metabolism of adrenal androgens is the only source)

- over 60% of plasma estrogens are bound to albumin, the rest is bound to SHBG

- estrogens stimulate the synthesis of:

  - SHBG and TBG and other binding proteins

- in normal male: estrogen present at low conc. in plasma
  - from testis
  - and from metabolism of T in liver & adipose tissue

Progesterone

- secreted only from corpus luteum and placenta
- it is also an intermediate in hormone biosynthesis

- in plasma mostly bound to albumin (and also to transcortin)

- it effects uterus: on the implantation of the fetus in endometrium

- its measurement in plasma for the investigation of infertility

SHBG

- binds both T & E_2 (greater affinity to T)
- plasma conc.of SHBG in males is half that in females
- factors that alter SHBG conc., will alter the ratio of free T to free E_2 :

  *if SHBG conc. Decreased: the ratio of free T / free E_2 increase

  *if SHBG conc. Increase, the ratio decrease

- thus in either sex: the effect of an increase in SHBG is to increase estrogenic effects , while a decrease in SHBG increase androgenic effects

Factors affecting sex hormone-binding globulin concentration

Increase

oestrogens
hyperthyroidism
liver cirrhosis

Decrease

androgens
hypothyroidism
glucocorticoids
malnutrition and malabsorption
protein-losing states
obesity, particularly in women

Disorders of gonadal function

in Male

Delayed puberty and hypogonadism

- It can be:

-idiopathic (family history)

-related to chronic illness (cystic fibrosis)

-or as a result of hypogonadism

- Hypogonadism is defective: spermatogenesis or testosterone production or both

- it could be: primary or secondary
- Biochemical investigation is to distinguish between primary and secondary hypogonadism

Some causes of male hypogonadism

Primary (serum testosterone ↓; FSH and LH ↑)

congenital
- testicular agenesis
- Klinefelter's syndrome (47XXY)
- 5α-reductase and other enzyme defects
- untreated cryptorchidism

acquired
- bilateral orchitis (mumps)
- bilateral testicular torsion
- irradiation
- cytotoxic drugs
- bilateral varicocele

Secondary (serum testosterone ↓; FSH and LH normal or ↓)

pituitary disorders
- tumours (especially if causing hyperprolactinaemia)
- panhypopituitarism

hypothalamic disorders
- Kallman's syndrome

Hypogonadism.. Cont..

- since the secretion of LH, FSH and T is pulsatile

- analyses should be performed on several blood specimens drawn over the period of an hour

Human chorionic gonadotrophin test	
Procedure	**Results**
day 0: 0900 h; take blood for testosterone; give 5000 U hCG i.m.	normal response: plasma testosterone level increases to above upper limit of reference range
day 4: 0900 h; take blood for testosterone	primary testicular failure: little or no response secondary testicular failure: response may be normal

A protocol for the (hCG) test for primary testicular failure

Gynecomastia

- brest development in male, due to:

  disturbance in the balance of estrogen to androgen

- investigations help to establish the cause.

  *it include the measurement of plasma:

  *T, E_2, gonadotropins, hCG, SHBG, prolactin

- test of functions of:
- (renal, thyroid, adrenal, and pituitary) functions

Some causes of gynaecomastia

Physiological

neonatal
pubertal
old age

Pathological

increased oestrogens
  chronic liver disease[a], end-stage renal failure, Cushing's syndrome, hyperthyroidism[a], tumours
decreased androgens
  Klinefelter's syndrome
androgen insensitivity
  testicular feminization
  re-feeding after starvation (LH secretion increased)

Pharmacological

oestrogens
digoxin (binds to oestrogen receptors)
cytotoxics (testicular damage)
anti-androgens (e.g. cyproterone; spironolactone has some anti-androgenic activity)
many others (phenothiazines, methyldopa etc; mechanism uncertain)

Disorders of gonadal function

In female

Delayed puberty and hypogonadism

- girls enter puberty near the age of 13
- delayed puberty appear as:
  - absence of brest development
  - and/or amenorrhea

- if this remains till the age of 15 years

- further investigations are required

Some causes of female hypogonadism

Primary (serum oestradiol ↓; FSH and LH ↑)

congenital

- Turner's syndrome (45XO) and variants
- Noonan's syndrome (46XX)

acquired

- chemotherapy/radiotherapy

Secondary (serum oestradiol ↓; FSH and LH normal or ↓)

see causes of secondary male hypogonadism (Fig. 10.3)

Amenorrhea and oligomenorrhea

- it can be primary or secondary
- the primary may occur :
  -as a part of female hypogonadism
  -or may occur in normally feminized women

- pregnancy → cause amenorrhea

- investigations include measurement of: LH, FSH, PRL

*high FSH → indicate ovarian failure

*elevated LH → mostly diagnosed as polycystic ovarian syndrome (PCOS)

- if LH and FSH are normal or low

- a pituitary or hypothalamic disorder should considered

Climacteric

- during this period progressive ovarian failure

- causes decline in E_2 secretion → menestruation stops

- plasma LH and FSH greatly elevated

- metabolic changes after menopause occur include:

  -increase in plasma LDL and urate conc.

  -osteoporosis is mostly due to E_2 deficiency of post-menopause

Hirsutism and virilism

- virilism is male pattern: male character

- hirsutism: increased body hair due to excessive exposure of tissues to androgen either due to

  -increased production of androgen

  -or low levels of SHBG which increase free testosterone fraction

- investigations: measurement of:

  LH, FSH, T, and SHBG

Causes of hirsutism and virilization

Idiopathic

Ovarian

polycystic ovary syndrome
androgen-secreting tumours
post-menopausal

Adrenal

congenital adrenal hyperplasia
Cushing's syndrome
androgen-secreting tumours

Iatrogenic

androgens
progestogens

infertility

- a common clinical problem
- it can be: primary or secondary affecting either male or female

- mostly due to:

*hyperprolactinemia

*hypothalamic-pituitary dysfunction (LH, FSH problems)

*defective sperm production

- Lab investigation are essential to follow ovulation by measurement of progesterone which increases after ovulation :

- measurement of PRL and gonadotropins may help in diagnosis

- defective sperm production is investigated by measurement of T and gonadotropins

Pregnancy: hormonal changes

- hCG : produced by the newly developed placenta
- it can be detected in plasma of pregnant woman 7-9 days after conception and in urine 2 days later
- its detection in urine is highly sensitive pregnancy test
- hCG also produced by some tumors: so it can be used as tumor marker
- ectopic pregnancy can be diagnosed by measurement of hCG

Pregnancy .. Cont..

- estrogens: after 6 weeks of pregnancy, placenta is the major source of estrogens:

  (E_2, estrone and estriol)

- estriol is produced in massive amounts from placenta from the metabolism of fetal adrenal gland

- other placental products:
- human placental lactogen
- placental ALP

- Patients during pregnancy may require close monitoring e.g:

  - strict control of diabetes mellitus is vital for both maternal and fetal health

  - glycated hemoglobin + blood glucose measurement

- Urine should be tested for :

  - proteinuria (an early sign of pregnancy-induced hypertension and edema) ,{ previously“pre-eclampsia” }
  - glycosuria (renal threshold for glucose is decreased during pregnancy)

Metabolic changes during pregnancy and use of oral contraceptives

Change	Cause	Pregnancy	Oral contraceptive use
↓ urea	↑ GFR; ↑ plasma volume	*	
↓ albumin	↑ plasma volume	*	
↓ total protein	↑ plasma volume	*	
↑ total thyroxine	↑ TBG	*	*
↑ cortisol	↑ transcortin	*	*
↑ copper	↑ caeruloplasmin	*	*
glycosuria	↓ renal threshold	*	
↓ glucose tolerance (but normal fasting concentrations)		*	
↑ triglyceride (VLDL) ↑ LDL cholesterol ↑ HDL cholesterol	↑ oestrogens (antagonism of actions of insulin)	* * *see legend*	* variable variable
↑ alkaline phosphatase	placental isoenzyme	*	