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 undergo hydration to form a weak acid: carbonic acid.

\[ \text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \]

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$_2$CO$_3$).
- 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 \((\text{H}_2\text{CO}_3)\) can dissociate to water and \(\text{CO}_2\) allowing \(\text{CO}_2\) to be eliminated by lung
  - Changes in \(\text{CO}_2\) modify the ventilation rate
  - \(\text{HCO}_3^-\) concentration can be altered by the kidney

- Phosphate buffer system \((\text{HPO}_4^{2-} - \text{H}_2\text{PO}_4^-)\) plays a role in plasma and RBC’s and is involved in the exchange of \(\text{Na}/\text{H}^+\) ion in the urine filtrate

- Plasma proteins, forms important buffer system in plasma. Most circulating proteins has net negative charge capable of \(\text{H}^+\) 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**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.35–7.45</td>
</tr>
<tr>
<td>PCO₂ (mm Hg)</td>
<td>35–45</td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/L)</td>
<td>22–26</td>
</tr>
<tr>
<td>Total CO₂ content (mmol/L)</td>
<td>23–27</td>
</tr>
<tr>
<td>PO₂ (mm Hg)</td>
<td>80–110</td>
</tr>
<tr>
<td>SO₂ (%)</td>
<td>&gt;95</td>
</tr>
<tr>
<td>O₂Hb (%)</td>
<td>&gt;95</td>
</tr>
</tbody>
</table>
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 $\beta$-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+HCO₃)
## Causes of lactic acidosis

<table>
<thead>
<tr>
<th>Causes of lactic acidosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tissue hypoxia</strong></td>
</tr>
<tr>
<td>decreased perfusion</td>
</tr>
<tr>
<td>reduced arterial $PO_2$</td>
</tr>
<tr>
<td><strong>Drugs etc.</strong></td>
</tr>
<tr>
<td>ethanol, methanol</td>
</tr>
<tr>
<td>phenformin</td>
</tr>
<tr>
<td>fructose, sorbitol</td>
</tr>
<tr>
<td><strong>Congenital</strong></td>
</tr>
<tr>
<td>glucose 6-phosphatase deficiency</td>
</tr>
<tr>
<td>other inherited diseases with defective</td>
</tr>
<tr>
<td>gluconeogenesis or pyruvate oxidation</td>
</tr>
</tbody>
</table>
Fig. 3 Reasons for metabolic acidosis and alkalosis.

- Impaired H⁺ excretion
- Increased H⁺ production or ingestion
- Loss of HCO₃⁻

ACIDOSIS

- Loss of H⁺ in vomit
- Alkali ingestion
- Potassium deficiency

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 $\text{HCO}_3^-$ concentration is balanced by increased $\text{Cl}$ 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

<table>
<thead>
<tr>
<th>Principal causes of non-respiratory acidosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Increased $H^+$ formation</strong></td>
</tr>
<tr>
<td>ketoacidosis (usually diabetic, also alcoholic)</td>
</tr>
<tr>
<td>lactic acidosis</td>
</tr>
<tr>
<td>poisoning, e.g. ethanol, methanol, ethylene glycol and salicylate</td>
</tr>
<tr>
<td>inherited organic acidoses</td>
</tr>
<tr>
<td><strong>Acid ingestion</strong></td>
</tr>
<tr>
<td>acid poisoning</td>
</tr>
<tr>
<td>excessive parenteral administration of amino acids, e.g. arginine, lysine and histidine</td>
</tr>
<tr>
<td><strong>Decreased $H^+$ excretion</strong></td>
</tr>
<tr>
<td>renal tubular acidoses (types 1 and 4)</td>
</tr>
<tr>
<td>generalized renal failure</td>
</tr>
<tr>
<td>carbonate dehydratase inhibitors</td>
</tr>
<tr>
<td><strong>Loss of bicarbonate</strong></td>
</tr>
<tr>
<td>diarrhoea</td>
</tr>
<tr>
<td>ileostomy</td>
</tr>
<tr>
<td>pancreatic, intestinal and biliary fistulae or drainage</td>
</tr>
<tr>
<td>renal tubular acidosis (type 2)</td>
</tr>
</tbody>
</table>

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.
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

<table>
<thead>
<tr>
<th>Principal causes of non-respiratory alkalosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primarily related to volume depletion/hypochloraemia</strong></td>
</tr>
<tr>
<td>gastrointestinal</td>
</tr>
<tr>
<td>gastric aspiration</td>
</tr>
<tr>
<td>vomiting with pyloric stenosis</td>
</tr>
<tr>
<td>congenital chloride-losing diarrhoea</td>
</tr>
<tr>
<td>renal</td>
</tr>
<tr>
<td>diuretic therapy (not K⁺-sparing)</td>
</tr>
<tr>
<td><strong>Primarily related to potassium depletion</strong></td>
</tr>
<tr>
<td>inadequate intake</td>
</tr>
<tr>
<td>increased excretion</td>
</tr>
<tr>
<td>mineralocorticoid excess</td>
</tr>
<tr>
<td>Conn’s syndrome (primary aldosteronism)</td>
</tr>
<tr>
<td>Cushing’s syndrome</td>
</tr>
<tr>
<td>Barter’s syndrome and related conditions</td>
</tr>
<tr>
<td>(see Figs 2.18 and 14.15)</td>
</tr>
<tr>
<td>secondary aldosteronism</td>
</tr>
<tr>
<td>drugs increasing mineralocorticoid activity, carbenoxolone</td>
</tr>
<tr>
<td><strong>Administration of alkali</strong></td>
</tr>
<tr>
<td>inappropriate treatment of acidotic states</td>
</tr>
<tr>
<td>chronic alkali ingestion</td>
</tr>
</tbody>
</table>

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 CO₂ is not effectively removed from the blood. In certain patients with chronic obstructive pulmonary disease (COPD), where CO₂ is retained in the blood, causing chronic hypercardia (elevated PCO₂).

- 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 PCO₂ 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 PCO₂.

- 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

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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 HCO₃⁻ 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*

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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
Cortisol.. Cont..

• measurement of urinary cortisol is valuable in investigating Cushing’s syndrome

• 95% is bound to Corticosteroids Binding Globulin { CBG } (transcortin)

• Transcortin 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

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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
<table>
<thead>
<tr>
<th>Causes</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Common</strong></td>
<td>Common</td>
</tr>
<tr>
<td>glucocorticoid treatment</td>
<td>tiredness,</td>
</tr>
<tr>
<td>autoimmune adrenalitis</td>
<td>generalized weakness, lethargy</td>
</tr>
<tr>
<td>tuberculosis</td>
<td>anorexia, nausea, vomiting</td>
</tr>
<tr>
<td></td>
<td>weight loss</td>
</tr>
<tr>
<td></td>
<td>dizziness and postural hypotension</td>
</tr>
<tr>
<td></td>
<td>pigmentation</td>
</tr>
<tr>
<td></td>
<td>loss of body hair (women)</td>
</tr>
<tr>
<td><strong>Less common</strong></td>
<td>Less common</td>
</tr>
<tr>
<td>adrenalectomy</td>
<td>hypoglycaemia</td>
</tr>
<tr>
<td>secondary tumour deposits</td>
<td>depression</td>
</tr>
<tr>
<td>amyloidosis</td>
<td></td>
</tr>
<tr>
<td>haemochromatosis</td>
<td></td>
</tr>
<tr>
<td>histoplasmosis</td>
<td></td>
</tr>
<tr>
<td>adrenal haemorrhage</td>
<td></td>
</tr>
</tbody>
</table>
Causes of adrenocortical insufficiency

Fig. 2 Causes of adrenocortical insufficiency.
## ACTH stimulation tests

<table>
<thead>
<tr>
<th>Short test</th>
<th>Long test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Procedure</strong></td>
<td><strong>Procedure</strong></td>
</tr>
<tr>
<td>take blood sample at 0900 h for measurement of cortisol</td>
<td>0900 h: take blood for measurement of cortisol</td>
</tr>
<tr>
<td>inject 250 μg ACTH i.m. or i.v.</td>
<td>inject 1 mg depot ACTH i.m.</td>
</tr>
<tr>
<td>take further blood samples after 30 and 60 min for cortisol measurement</td>
<td>1500 h: take blood for cortisol measurement</td>
</tr>
<tr>
<td><strong>Normal results</strong></td>
<td><strong>Results</strong></td>
</tr>
<tr>
<td>plasma cortisol after ACTH</td>
<td>primary adrenal insufficiency: no increase in cortisol</td>
</tr>
<tr>
<td>increment of 200 nmol/L with peak of &gt;550 nmol/L</td>
<td>secondary adrenal insufficiency: increase in cortisol</td>
</tr>
</tbody>
</table>

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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

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal result</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h urinary cortisol excretion</td>
<td>&lt;300 nmol/24 h</td>
</tr>
<tr>
<td>overnight/48 h low-dose dexamethasone suppression test</td>
<td>plasma cortisol &lt;50 nmol/L at 0900 h</td>
</tr>
</tbody>
</table>
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
- bilateral hypertrophy of zona glomerulosa cells
- 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
- 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
  \[ \downarrow \]
  - which causes increased ACTH secretion
  \[ \downarrow \]
  - this will cause hyperplasia of the gland
  \[ \downarrow \]
  - results in accumulation of 17α-hydroxyprogesterone
    \[ \downarrow \]
    - results in increased formation of adrenal androgen

In female: at birth
- With ambiguous genitalia

In male: pseudo puberty in their 2\textsuperscript{nd} or 3\textsuperscript{rd} year of life
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
• Partial deficiency in 11β-hydroxylase is more common than complete deficiency of the enzyme

↓

• accumulation of 11-deoxycorticosterone (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

[Diagram showing the metabolism of catecholamines, including noradrenaline, normetadrenaline, sulphate and glucuronide conjugates, dihydroxymandelic acid, HMMA, monoamine oxidase, and COMT pathways.]

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Disorders of the adrenal medulla

- the main disorder that is of interest to clinical biochemistry is: **pheochromocytomas**
- are tumors that secret catecholamines
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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Lectures in Clinical Chemistry
Department of Pharmacy
Alnoor University College
| 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 exchange in the body

- Bone: 25,000 mmol (100 mmol rapidly exchangeable)
- Exchange: 500 mmol/24 h
- Bone formation: 7.5 mmol/24 h
- Bone resorption: 7.5 mmol/24 h
- Extracellular fluid (ECF): 22.5 mmol
- Plasma: 9.0 mmol
- Absorption: 12 mmol/24 h
- Secretion: 6 mmol/24 h
- Glomerular filtration: 240 mmol/24 h
- Reabsorption: 234 mmol/24 h
- Urine: 6 mmol/24 h
- Faeces: 19 mmol/24 h

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Distribution of calcium in human plasma

- Total calcium: 2.25–2.60 mmol/L
  - Diffusible: 54%
  - Non-diffusible (protein bound): 46%
  - Free (physiologically active): 47%
  - Complexed (with citrate, phosphate): 7%
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 24 hours
• 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
• due to increased resorption of bone, so in kidneys: PTH increases filtered amount of calcium

\[ \downarrow \]

• 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

<table>
<thead>
<tr>
<th>Target organ</th>
<th>Action</th>
<th>Effect</th>
</tr>
</thead>
</table>
| bone         | rapid release of calcium  
  ↑ osteoclastic resorption | ↑ plasma [Ca\(^+\)] |
| kidney       | ↑ calcium reabsorption  
  ↓ phosphate reabsorption  
  ↑ 1α-hydroxylation of  
  25-hydroxycholecalciferol  
  ↓ bicarbonate reabsorption | ↑ plasma [Ca\(^+\)]  
  ↓ plasma [Pi]  
  ↑ calcium and phosphate absorption from gut  
  acidosis |
Calcitriol: actions and control of renal synthesis
Homeostatic response to hypocalcemia
Homeostatic responses in hypophosphatemia
Disorders of calcium, phosphate, and magnesium metabolism
<table>
<thead>
<tr>
<th>Causes</th>
<th>Clinical features</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Common</strong></td>
<td>weakness, tiredness, lassitude, weight loss and muscle weakness</td>
</tr>
<tr>
<td>malignant disease, with or without metastasis to bone primary hyperparathyroidism</td>
<td>mental changes (impaired concentration, drowsiness, personality changes, coma)</td>
</tr>
<tr>
<td><strong>Less common</strong></td>
<td>anorexia, nausea, vomiting and constipation</td>
</tr>
<tr>
<td>thyrotoxicosis</td>
<td>abdominal pain (rarely peptic ulceration and pancreatitis)</td>
</tr>
<tr>
<td>vitamin D intoxication</td>
<td>polyuria, dehydration and renal failure</td>
</tr>
<tr>
<td>thiazide diuretics</td>
<td>renal calculi and nephrocalcinosis</td>
</tr>
<tr>
<td>sarcoidosis</td>
<td>(mainly associated with primary hyperparathyroidism)</td>
</tr>
<tr>
<td>familial hypocalciuric hypercalcaemia</td>
<td>short QT interval on ECG</td>
</tr>
<tr>
<td>renal transplantation (tertiary hyperparathyroidism)</td>
<td>cardiac arrhythmias and hypertension</td>
</tr>
<tr>
<td><strong>Uncommon</strong></td>
<td>corneal calcification and vascular calcification</td>
</tr>
<tr>
<td>milk–alkali syndrome</td>
<td>there may also be features of the underlying disorder, such as bone pain in malignant disease and hyperparathyroidism</td>
</tr>
<tr>
<td>lithium treatment</td>
<td></td>
</tr>
<tr>
<td>tuberculosis</td>
<td></td>
</tr>
<tr>
<td>immobilization (especially in Paget’s disease)</td>
<td></td>
</tr>
<tr>
<td>acute adrenal failure</td>
<td></td>
</tr>
<tr>
<td>idiopathic hypercalcaemia of infancy</td>
<td></td>
</tr>
<tr>
<td>diuretic phase of acute renal failure</td>
<td></td>
</tr>
</tbody>
</table>

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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 Hypocaliuric Hypercalcemia
  – Benign condition
  – Germline mutation of CSR (calcium sensing receptor)
  – Stable, mild hypercalcemia from birth
  – Hypocalciuria
### Hypocalcemia

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

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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 opposite of these states

  PTH and aldosterone: both affect the renal handling of magnesium

  Hypermagnesemia : is uncommon
### 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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Alnoor University College
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

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Target organ</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>growth hormone (GH)</td>
<td>liver</td>
<td>somatomedin synthesis, hence growth stimulation metabolic regulation</td>
</tr>
<tr>
<td></td>
<td>others</td>
<td></td>
</tr>
<tr>
<td>prolactin</td>
<td>breast</td>
<td>lactation</td>
</tr>
<tr>
<td>thyroid-stimulating hormone (TSH)</td>
<td>thyroid</td>
<td>thyroid hormone synthesis and release</td>
</tr>
<tr>
<td>follicle-stimulating hormone (FSH)</td>
<td>ovary</td>
<td>oestrogen synthesis oogenesis</td>
</tr>
<tr>
<td></td>
<td>testis</td>
<td>spermatogenesis</td>
</tr>
<tr>
<td>luteinizing hormone (LH)</td>
<td>ovary</td>
<td>ovulation corpus luteum, hence progesterone production testosterone synthesis</td>
</tr>
<tr>
<td></td>
<td>testis</td>
<td></td>
</tr>
<tr>
<td>adrenocorticotropic hormone (ACTH)</td>
<td>adrenal cortex</td>
<td>glucocorticoid synthesis and release</td>
</tr>
<tr>
<td></td>
<td>skin</td>
<td>pigmentation</td>
</tr>
<tr>
<td>β-lipotrophin</td>
<td></td>
<td>precursor of endorphins</td>
</tr>
</tbody>
</table>
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 hypothalamic
  *somatostatin 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
• 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 T₄ 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 $\alpha$ and $\beta$ subunits
- $\alpha$-subunit is similar in TSH and gonadotropins, but the $\beta$-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
Figure 2: Outline of the hypothalamic-pituitary-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 (pathological 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 controlled 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 resemble 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 → cause increased secretion of cortisol by adrenal cortex
  - hyperpigmentation → due to increased ACTH
Clinical features of excessive GH secretion

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

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# Hyperprolactinemia

## 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

### Ectopic secretion
- bronchial carcinomas
- other tumours, e.g. thymus and prostate

### Inappropriate secretion
- pulmonary diseases
  - pneumonia
  - tuberculosis
  - positive pressure mechanical ventilation
- cerebral diseases
  - head injury
  - encephalitis
  - tumours
  - aneurysms
- miscellaneous
  - pain, e.g. postoperative
  - acute intermittent porphyria
  - Guillain–Barré syndrome
  - hypothyroidism
- drugs, e.g. narcotics, chlorpropamide,
  - carbamazepine, oxytocin and vinca alkaloids
Diabetes insipidus

• decreased secretion of ADH:
  - results in diabetes insipidus which is
    uncontrolled excretion of water with a tendency of severe 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
- 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

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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)

patient empties bladder: measure urine volume and osmolality

measure plasma osmolality (stop test if osmolality >300 mmol/kg)

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

Dr. Nabeel Nuaimi
Lectures in Clinical Chemistry
Department of Pharmacy
Alnoor University College
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 ..)
<table>
<thead>
<tr>
<th>Apolipoprotein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-I</td>
<td>activates LCAT structural (in HDL)</td>
</tr>
<tr>
<td>A-II</td>
<td>inhibits HTGL at high concentration structural (in HDL)</td>
</tr>
<tr>
<td>B-100</td>
<td>structural (in LDL and VLDL) receptor binding</td>
</tr>
<tr>
<td>B-48</td>
<td>structural (in chylomicrons)</td>
</tr>
<tr>
<td>C-II</td>
<td>activator of LPL</td>
</tr>
<tr>
<td>C-III</td>
<td>inhibits LPL inhibits clearance of CM and VLDL remnant particles</td>
</tr>
<tr>
<td>E</td>
<td>binding to LDL and remnant receptors</td>
</tr>
</tbody>
</table>

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● 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
<table>
<thead>
<tr>
<th>Lipoprotein Type</th>
<th>Triglyceride</th>
<th>Cholesterol</th>
<th>Phospholipid</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicrons</td>
<td>5%</td>
<td>4%</td>
<td>1%</td>
<td></td>
</tr>
<tr>
<td>VLDL</td>
<td>20%</td>
<td>15%</td>
<td>30%</td>
<td>25%</td>
</tr>
<tr>
<td>IDL</td>
<td>35%</td>
<td>20%</td>
<td>10%</td>
<td>5%</td>
</tr>
<tr>
<td>LDL</td>
<td>20%</td>
<td>20%</td>
<td>10%</td>
<td>15%</td>
</tr>
<tr>
<td>HDL</td>
<td>5%</td>
<td>55%</td>
<td>15%</td>
<td></td>
</tr>
</tbody>
</table>

Apolipoproteins:
- Chylomicrons: C, B-48, E, A
- VLDL: B-100, C, E
- IDL: B-100, E
- LDL: B-100
- HDL: A, C, E
<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Density (g/mL)</th>
<th>Mean diameter (nm)</th>
<th>Electrophoretic mobility</th>
<th>Source</th>
<th>Principal function</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM</td>
<td>&lt;0.95</td>
<td>500</td>
<td>remains at origin</td>
<td>intestine</td>
<td>transport of exogenous triglyceride</td>
</tr>
<tr>
<td>VLDL</td>
<td>0.96–1.006</td>
<td>43</td>
<td>pre-β</td>
<td>liver</td>
<td>transport of endogenous triglyceride</td>
</tr>
<tr>
<td>IDL</td>
<td>1.007–1.019</td>
<td>27</td>
<td>‘broad β’</td>
<td>catabolism of VLDL</td>
<td>precursor of LDL</td>
</tr>
<tr>
<td>LDL</td>
<td>1.02–1.063</td>
<td>22</td>
<td>β</td>
<td>catabolism of VLDL, via IDL</td>
<td>cholesterol transport</td>
</tr>
<tr>
<td>HDL</td>
<td>1.064–1.21</td>
<td>8</td>
<td>α</td>
<td>liver, intestine; catabolism of CM and VLDL</td>
<td>reverse cholesterol transport</td>
</tr>
</tbody>
</table>
Chylomicrons metabolism
LDL uptake and catabolism
Uptake of modified LDL by macrophages

scavenger receptors

• macrophages possess 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 downregulated 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 $\rightarrow$ 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

• hypertriglycerideridemia 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

![Graph showing the relationship between plasma cholesterol concentration (mmol/L) and CHD risk ratio for smokers and non-smokers. The graph indicates a higher risk ratio for smokers across the range of cholesterol concentrations.]
<table>
<thead>
<tr>
<th>Variable</th>
<th>HDL cholesterol</th>
<th>LDL cholesterol</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex</td>
<td>F &gt; M</td>
<td>M = F</td>
<td>F &lt; M</td>
</tr>
<tr>
<td>age</td>
<td>slight ↑ in F</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>high P: S ratio</td>
<td>N or ↓</td>
<td>↓</td>
<td>N or ↓</td>
</tr>
<tr>
<td>exercise</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>obesity</td>
<td>↓</td>
<td>N</td>
<td>↑</td>
</tr>
<tr>
<td>alcohol</td>
<td>↑</td>
<td>N</td>
<td>↑</td>
</tr>
<tr>
<td>exogenous oestrogens</td>
<td>↑</td>
<td>↓</td>
<td>↑°</td>
</tr>
</tbody>
</table>
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 arteriosclerotic
Common causes of secondary hyperlipidemia

<table>
<thead>
<tr>
<th>Condition</th>
<th>HDL cholesterol</th>
<th>LDL cholesterol</th>
<th>Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>obesity</td>
<td>↓</td>
<td>N</td>
<td>↑</td>
</tr>
<tr>
<td>excessive alcohol intake</td>
<td>↑</td>
<td>N</td>
<td>↑</td>
</tr>
<tr>
<td>diabetes mellitus</td>
<td>N/sl ↓</td>
<td>N</td>
<td>↑↑</td>
</tr>
<tr>
<td>hypothyroidism</td>
<td>N</td>
<td>↑↑</td>
<td>N/↑</td>
</tr>
<tr>
<td>nephrotic syndrome</td>
<td>↓</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>chronic renal failure</td>
<td>↓</td>
<td>N/↑</td>
<td>↑</td>
</tr>
<tr>
<td>cholestasis</td>
<td>N</td>
<td>↑</td>
<td>N</td>
</tr>
</tbody>
</table>
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: hypoalphalipoproteinemia & hypobetalipoproteinemia

• 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.
<table>
<thead>
<tr>
<th>Modifiable</th>
<th>Not modifiable</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypercholesterolaemia(^a)</td>
<td>personal history of cardiovascular</td>
</tr>
<tr>
<td>hypertension(^a)</td>
<td>disease(^a)</td>
</tr>
<tr>
<td>cigarette smoking(^a)</td>
<td>family history of premature</td>
</tr>
<tr>
<td>diabetes mellitus(^a)</td>
<td>cardiovascular disease(^a)</td>
</tr>
<tr>
<td>hyperfibrinogenenaemia</td>
<td></td>
</tr>
<tr>
<td>hyperhomocysteinaemia</td>
<td></td>
</tr>
<tr>
<td>low HDL cholesterol</td>
<td></td>
</tr>
<tr>
<td>hypertriglyceridaemia</td>
<td></td>
</tr>
<tr>
<td>overweight</td>
<td></td>
</tr>
<tr>
<td>sedentary lifestyle etc.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>male sex(^a)</td>
</tr>
<tr>
<td></td>
<td>age(^a)</td>
</tr>
</tbody>
</table>

\(^a\) Indicates factors that can be modified through lifestyle changes.
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
  \begin{itemize}
  \item ANP
  \item BNP
  \end{itemize}

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

• a normal value\(\Rightarrow\) exclude heart failure

• other lab investigations may be necessary:
  plasma: albumin, creatinine and potassium
<table>
<thead>
<tr>
<th>Investigation</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>full blood count</td>
<td>anaemia is a cause of ‘high output’ heart failure</td>
</tr>
<tr>
<td>plasma albumin</td>
<td>hypoalbuminaemia is another cause of oedema</td>
</tr>
<tr>
<td>plasma creatinine</td>
<td>renal impairment</td>
</tr>
<tr>
<td>plasma potassium</td>
<td>diuretic-induced hypokalaemia</td>
</tr>
<tr>
<td>thyroid function tests&lt;sup&gt;a&lt;/sup&gt;</td>
<td>thyrotoxicosis</td>
</tr>
<tr>
<td>plasma ferritin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>haemochromatosis</td>
</tr>
<tr>
<td>serum and urine electrophoresis&lt;sup&gt;a&lt;/sup&gt;</td>
<td>myeloma-related amyloid</td>
</tr>
<tr>
<td>endocrine causes of hypertension&lt;sup&gt;a&lt;/sup&gt;</td>
<td>see Figure 14.16</td>
</tr>
</tbody>
</table>


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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
<table>
<thead>
<tr>
<th>Investigation</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>urine analysis</td>
<td>for protein (renal disease) and glucose (diabetes, a cardiovascular risk factor)</td>
</tr>
<tr>
<td>plasma creatinine</td>
<td>renal disease</td>
</tr>
<tr>
<td>plasma potassium</td>
<td>mineralocorticoid excess (primary or secondary)</td>
</tr>
<tr>
<td>plasma calcium</td>
<td>hyperparathyroidism</td>
</tr>
<tr>
<td>plasma cholesterol and triglycerides</td>
<td>cardiovascular risk assessment</td>
</tr>
<tr>
<td>plasma renin and aldosterone&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Conn’s syndrome</td>
</tr>
<tr>
<td>overnight dexamethasone suppression test&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cushing’s syndrome</td>
</tr>
<tr>
<td>urinary catecholamines/metabolites&lt;sup&gt;a&lt;/sup&gt;</td>
<td>phaeochromocytoma</td>
</tr>
</tbody>
</table>
Disorders of carbohydrate metabolism

Hyperglycemia
Hypoglycemia

Dr. Nabeel Nuaimi
Department of Pharmacy
Alnoor University College
• 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
<table>
<thead>
<tr>
<th>Hormone</th>
<th>Principal actions</th>
<th>M, A</th>
</tr>
</thead>
<tbody>
<tr>
<td>insulin</td>
<td>increases</td>
<td>L, M</td>
</tr>
<tr>
<td></td>
<td>sugar glucose uptake</td>
<td>L, M</td>
</tr>
<tr>
<td></td>
<td>protein synthesis</td>
<td>L, A</td>
</tr>
<tr>
<td></td>
<td>fatty acid and triglyceride synthesis</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>decreases</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>gluconeogenesis</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>glycogenolysis</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>ketogenesis</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>lipolysis</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>proteolysis</td>
<td></td>
</tr>
<tr>
<td>glucagon</td>
<td>increases</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>glycogenolysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>gluconeogenesis</td>
<td></td>
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<tr>
<td></td>
<td>ketogenesis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>lipolysis</td>
<td></td>
</tr>
<tr>
<td>adrenaline (epinephrine)</td>
<td>increases</td>
<td>L, M</td>
</tr>
<tr>
<td></td>
<td>glycogenolysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>lipolysis</td>
<td></td>
</tr>
<tr>
<td>growth hormone</td>
<td>increases</td>
<td></td>
</tr>
<tr>
<td></td>
<td>glycogenolysis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>lipolysis</td>
<td></td>
</tr>
<tr>
<td>cortisol</td>
<td>increases</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>gluconeogenesis</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>glycogen synthesis</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>proteolysis</td>
<td>L</td>
</tr>
<tr>
<td></td>
<td>decreases</td>
<td>L, M, A</td>
</tr>
<tr>
<td></td>
<td>tissue glucose utilization</td>
<td>A</td>
</tr>
</tbody>
</table>
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

C-peptide

proinsulin

insulin

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Hormonal regulation of glycolysis

- 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 insulin and glucagon on the synthesis of key enzymes of glycolysis in liver.
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 concentration of glucose in liver is elevated
- it has high $V_{max}$, removing the flood of glucose, thus minimizing hyperglycemia during absorptive period

Effect of glucose concentration on the rate of phosphorylation catalyzed by hexokinase and glucokinase
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).
- Type 2; (NIDDM); Non insulin dependent.
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/m²)
    • 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 methods.
  – 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**

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

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Role of Laboratory (cont’d)

• Glycosylated Hemoglobin/Hemoglobin A\textsubscript{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\textsubscript{1c} (HbA\textsubscript{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, unusual stress.
- Dehydration, ketosis, hyperventilation.
- Ketoacidosis may be due to:
  1. Insulin deficiency.
  2. High regulatory hormones (adrenalin, cortisol, growth hormone, glucagon).
  3. Hyperglycemia.
4- free fatty 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.
• NaHCO3 (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 hyperglycemic 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 considered.
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

Dr. Nabeel Nuaimi
Department of Pharmacy
Alnoor University College
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

<table>
<thead>
<tr>
<th>Specimen Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Venous blood, serum or plasma</td>
</tr>
<tr>
<td>Arterial blood</td>
</tr>
<tr>
<td>Capillary blood</td>
</tr>
<tr>
<td>Urine</td>
</tr>
<tr>
<td>Faeces</td>
</tr>
<tr>
<td>Cerebrospinal fluid (CSF)</td>
</tr>
<tr>
<td>Sputum and saliva</td>
</tr>
<tr>
<td>Tissue and cells</td>
</tr>
<tr>
<td>Aspirates, e.g., pleural fluid</td>
</tr>
<tr>
<td>ascites</td>
</tr>
<tr>
<td>joint (synovial) fluid</td>
</tr>
<tr>
<td>intestinal (duodenal)</td>
</tr>
<tr>
<td>pancreatic pseudocysts</td>
</tr>
<tr>
<td>Calculi (stones)</td>
</tr>
</tbody>
</table>
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)

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

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

1. 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

<table>
<thead>
<tr>
<th>Factor</th>
<th>Example of variable affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>alkaline phosphatase, urate</td>
</tr>
<tr>
<td>sex</td>
<td>gonadal steroids</td>
</tr>
<tr>
<td>ethnicity</td>
<td>creatine kinase</td>
</tr>
<tr>
<td>pregnancy</td>
<td>urea</td>
</tr>
<tr>
<td>posture</td>
<td>proteins</td>
</tr>
<tr>
<td>exercise</td>
<td>creatine kinase</td>
</tr>
<tr>
<td>stress</td>
<td>prolactin</td>
</tr>
<tr>
<td>nutritional status</td>
<td>glucose</td>
</tr>
<tr>
<td>time</td>
<td>cortisol</td>
</tr>
<tr>
<td>drugs</td>
<td>triglycerides (alcohol) γ-glutamyl transferase (phenytoin)</td>
</tr>
</tbody>
</table>
• 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
أحمد يحيى أحمد 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, NH4 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.
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
- OGT T (mg /dl)          <140      140-199     ≥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, hypopituitarism, 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 $\rightarrow$ Gluconate + H2O2

2H2O2 + phenol + 4-amino-antipyrine $\rightarrow$ Quinoneimine + 4H2O (pink dye)

**Procedure (Kit method) (BIOLABO)**

Pipette into test tubes

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Std</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent (ml)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Standard/R3 (ml)</td>
<td>-</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>Sample (serum) (ml)</td>
<td>-</td>
<td>-</td>
<td>0.01</td>
</tr>
<tr>
<td>Distilled water (ml)</td>
<td>0.01</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*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 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 = \( \frac{A_{t-AB}}{A_{st-AB}} \times 100 \text{ mg/dl (5.55 mmol/L)} \)
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**
Measurement of serum Urea
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.

Protein

\[ \text{Proteolysis} \]

\[ \text{Amino acid} \]

\[ \text{Transamination & oxidative deamination} \]

\[ \text{Ammonia} \]

\[ \text{Enzymatic urea cycle} \]

\[ \text{Urea} \]
Urea is the major end product of amino acid catabolism, it is synthesized in the liver and most of urea is excreted in the urine (90%). So, urea is normally present in the 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.

\[
\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2\text{NH}_3 + \text{CO}_2
\]

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.

\[
\text{NH}_4^+ + \text{Salicylate} + \text{Hypochlorite} \quad \xrightarrow{\text{Alkaline medium}} \quad \text{Indophenols (Green color)}
\]
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:

<table>
<thead>
<tr>
<th></th>
<th>St</th>
<th>Test</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard solution (<strong>R2</strong>)</td>
<td>10 µl</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Sample (Serum)</td>
<td>--</td>
<td>10µl</td>
<td>--</td>
</tr>
<tr>
<td>Distilled water</td>
<td>--</td>
<td>--</td>
<td>10µl</td>
</tr>
<tr>
<td>Working solution (<strong>R3</strong>) (A2+A1)</td>
<td>1ml</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>(A1 Coloring reagents (Sodium Salicylate + Sodium Nitroprosside + Phosphate Buffer) + A2 Urease)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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:
Serum Conc. Of urea = \frac{AT-AB}{A_{St} - AB} \times \text{Conc. Of St}
(50 \text{ mg%})
(or 8.33 \text{ mmol/l})

Conversion factor:
\text{mmol/l} \times 6 = \text{mg%}
\text{mg%} \times 0.167 = \text{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-hydroxybenzene 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 +CO2 + H2O2

- H2O2 + Chromogen \( \xrightarrow{\text{Peroxidase}} \) quinoneimine (red color complex)

- (chromogen = Amino-antipyrine + Dichlorohydroxybenzene sulfonate)
Reagents  (Kit method by BIOLABO)

- **R1: Buffer pH 8**
  - Dichloro-hydroxybenzene 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:

  - R1: Buffer (Dichloro-hyroxybenzene sulfonate +Potassium hexacyano ferrate)  
    | T     | S     | B     |
    |-------|-------|-------|
    | 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:

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

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>25 µl</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Standard (25 µl)</td>
<td>---</td>
<td>25 µl</td>
<td>---</td>
</tr>
<tr>
<td>Distilled water</td>
<td>---</td>
<td>---</td>
<td>25 µl</td>
</tr>
<tr>
<td>working solution (R1+R2)</td>
<td>1 ml</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

\[ R1: (\text{Amino-2-methyl-popanol-1} + \text{Hydrochloric acid}) \] + \[ R2: (\text{O-cresolphthalein complexone (CPC)} + \text{Hydroxy-8-Quinoline} + \text{Hydrochloric acid}) \]

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 = \( \frac{A_t-AB}{Ast-AB} \times \) Standard Concentration

- Total Serum Calcium = \( \frac{A_t-AB}{Ast-AB} \times 10 \text{ mg/dl (2.5 mmol/L)} \)

- Normal range: 2.10 – 2.60 mmol/L or
- \( (8.5-10.5 \text{ mg/dl}). \)
Reagents

• **R1  Calcium Buffer**
  - Amino-2-methyl-popanol-1  1.7 mmol/L  pH 11
  - Hydrochloric acid  210 mmol/L

• **R2  Calcium dye**
  - O-cresolphthalein complexone (CPC)  78 miromol/L
  - Hydroxy-8-Quinoline  3.36 mmol/L
  - Hydrochloric acid  25 mmol/L

• **R3  Calcium standard**
  - Calcium  10 mg/dl (2.5 mmol/L)

• **Working solution**
  - Mix 1 volume **R1** and 1 volume **E2**. Reagents may also be added separately.
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 ions → Acid medium → phosphomolybdic complex molybdate
**Procedure:** (Kit method) *(BIOLABO)*

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>1 ml</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
<tr>
<td>D. Water</td>
<td>20 µl</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Standard</td>
<td>---</td>
<td>20 µl</td>
<td>---</td>
</tr>
<tr>
<td>Specimen</td>
<td>---</td>
<td>---</td>
<td>20 µl</td>
</tr>
</tbody>
</table>

*(Reagent: Ammonium molybdate + Sulfuric acid)*

Mix well, incubate for 2 minutes at room temperature.
Read standard and test absorbances at 340 nm against reagent blank.
Calculation::

Serum inorganic phosphorus = \( \frac{AT - AB}{A_{std} - AB} \times \text{Std Conc} \)

Serum inorganic phosphorus = \( \frac{AT - AB}{A_{std} - AB} \times 5 \text{ mg/dl (1.62 mmol/L)} \)

Normal range (Adults): 0.8-1.5 mmol/l \( (2.4-4.5 \text{ mg/dl}) \)
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) >1.4 mmol/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)\(^1\)(\textit{elevated levels of cholesterol products, especially LDL and apoB}), nephrotic syndrome, hypothyroidism, obstructive jaundice, Primary biliary cholangitis, (previously called primary biliary cirrhosis), (\textit{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 + H₂O → Cholesterol + Fatty acids (esterase)

• Cholesterol + O₂ → 4-Cholesten-3-One + H₂O₂ (Cholesterol Oxidase)

• 2H₂O₂ + 4-aminoantipyrine + Phenol → Red quinone + 4 H₂O (Peroxidase)

• 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:

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

- Mix and incubate for 5 minutes at 37°C or for 10 minutes at room temperature then apply the following calculations.
Calculation:

- mg% of Serum Cholesterol = \( \frac{\text{At-AB} \times \text{Conc. Of standard Ast-AB}}{\text{At-AB} \times 200 \text{ mg/dl}} \)

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

- Conversion factor:
  
  \[ \text{mmol/L} = \text{mg%} \times 0.0259 \]
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

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

Where \( U_{cr} \) = urine creatinine concentration

\( P_{cr} \) = 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

\[
\text{Corrected clearance} = \text{measured clearance} \times \frac{1.73}{A}
\]

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 picrate 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 $\xrightarrow{\text{Alkaline medium}}$ Creatinine picrate $\xrightarrow{\text{Orange colour}}$
Procedure: Kinetic method (BIOLABO)

- Let stand reagent and specimen at room temperature

<table>
<thead>
<tr>
<th></th>
<th>Test</th>
<th>Standard</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working reagent (R1+R2)</td>
<td>1000 µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Specimen (Serum)</td>
<td>100 µl</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Standard</td>
<td>---</td>
<td>100 µl</td>
<td>---</td>
</tr>
<tr>
<td>Distilled water</td>
<td>---</td>
<td>---</td>
<td>100 µl</td>
</tr>
</tbody>
</table>

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 = \( \frac{AT - AB}{A_{std} - AB} \times \text{Conc. of std.} \)
\[ \text{Conc. of std.} = 2 \text{ mg/dl (177 µmol/L)} \]

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 = \( \frac{AT - AB}{A_{std} - AB} \times \text{Conc. of std.} \times \text{X dilution factor} \)
\[ \text{Conc. of std.} = 2 \text{ mg/dl (177 µmol/L)} \]

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 = \( \frac{Ur \text{ Cr} \times Ur \text{ volume (ml)}}{Scr \times Time (min.)} \)

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**
  - Standard 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) (m²) = 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, 170 x 60 = 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 $\rightarrow$ diazotized sulfanilic acid.

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

T. bilirubin + diazotized sulfanilic acid $\rightarrow$ azobilirubin

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

Direct bilirubin + diazotized sulfanilic acid $\rightarrow$ azobilirubin

Read the absorbance of the assay azobilirubin at 550 nm using blank
**Procedure:** (Kit method) (BIOLABO)

<table>
<thead>
<tr>
<th>Pipette into well identified test tubes:</th>
<th>TOTAL BILIRUBIN</th>
<th>DIRECT BILIRUBIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blank</td>
<td>Assay</td>
</tr>
<tr>
<td>Reagent R1</td>
<td>1 mL</td>
<td>1 mL</td>
</tr>
<tr>
<td>Reagent R2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>50 μL</td>
<td></td>
</tr>
<tr>
<td>Reagent R3 (Nitrite)</td>
<td>50 μL</td>
<td></td>
</tr>
</tbody>
</table>

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. = \( A_T - A_B \times X \) Factor

Serum Direct Bilirubin Conc. = \( A_T - A_B \times X \) 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)
<table>
<thead>
<tr>
<th><strong>Reagents</strong></th>
<th><strong>Concentration</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>R1</strong> : Sulfanilic acid</td>
<td>30 mmol/l</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>150 mmol/l</td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>7 mmol/l</td>
</tr>
<tr>
<td><strong>R2</strong> : Sulfanilic acid</td>
<td>30 mmol/l</td>
</tr>
<tr>
<td>Hydrochloric acid</td>
<td>150 mmol/l</td>
</tr>
<tr>
<td><strong>R3</strong> : Sodium nitrite</td>
<td>20 mmol/l</td>
</tr>
</tbody>
</table>
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  $\xrightarrow{\text{Alkaline phosphatase}}$ phenol + phosphate

• pH 10

• Phenol + Amino-4-antipyrine +Potassium Ferricyanide $\rightarrow$ Red color

• The presence of sodium arsenate in the reagent stops the enzymatic reaction.
Procedure: (Kit method) (BIOLABO)

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

\[
\text{Alk. ph. Activity} = \frac{A_T - A_b}{A_{st} - A_b} \times n \quad \text{where } n = 20 \text{ kind and king unit/100ml}
\]

Alk. ph. Activity \(= 0.\ldots \times 20 = !!!! \text{ 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)

\[= 7.09 \times !!!! \]

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 antipyrene 60mmol/L
  • Sodium arsenate 240 mmol/L

• **R4: Dye reagent**
  • Potassium Ferricyanide 150 mmol/L
The Liver
Liver function Tests

Dr. Nabeel Nuaimi
Department of Pharmacy
Alnoor University College

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
• 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

**Carbohydrate metabolism**
gluconeogenesis
glycogen synthesis and breakdown

**Fat metabolism**
fatty acid synthesis
cholesterol synthesis and excretion
lipoprotein synthesis
ketogenesis
bile acid synthesis
25-hydroxylation of vitamin D

**Protein metabolism**
synthesis of plasma proteins (including some coagulation factors but not immunoglobulins)
urea synthesis from ammonia

**Hormone metabolism**
metabolism and excretion of steroid hormones
metabolism of polypeptide hormones

**Drugs and foreign compounds**
metabolism and excretion

**Storage**
glycogen
vitamin A
vitamin B₁₂
iron

**Metabolism and excretion of bilirubin**
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 urobilins & 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

➢ Urobilinogen 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

Hyperbilirubinemia:

- unconjugated (indirect)
- conjugated (direct)
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.
Hyperbilirubinemia may be caused by:
- Increased production
- Impaired metabolism
- Decreased excretion
- Combination of these

Jaundice is due to hyperbilirubinemia:
- Prehepatic
- Hepatocellular
- Cholestatic (post hepatic)
## Causes of jaundice

<table>
<thead>
<tr>
<th>Major causes of jaundice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-hepatic</strong></td>
</tr>
<tr>
<td>haemolysis</td>
</tr>
<tr>
<td>ineffective erythropoiesis</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Hepatic</strong></td>
</tr>
<tr>
<td>pre-microsomal</td>
</tr>
<tr>
<td>drugs, e.g. rifampicin, which interfere with bilirubin uptake</td>
</tr>
<tr>
<td>microsomal</td>
</tr>
<tr>
<td>prematurity</td>
</tr>
<tr>
<td>hepatitis, e.g. viral or drug-induced</td>
</tr>
<tr>
<td>Gilbert’s syndrome</td>
</tr>
<tr>
<td>Crigler–Najjar syndrome</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Prehepatic hyperbilirubinaemia;

Increased [unconjugated bil] due to;

• Hemolytic anemia
• Ineffective erythropoiesis e.g. pernicious anemia
• bleeding
Hepatocellular hyperbilirubinaemia

- Drugs or toxins
- Cirrhosis
- Low activity of UDP glucouronyltransferase in congenital deficiency (Gilberts syndrome & Crigler-Najjar syndrome)
- Premature infants
- Dubin-Jonson syndrome (benign)
# Inherited disorders of bilirubin metabolism

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

<table>
<thead>
<tr>
<th>Protein</th>
<th>Condition</th>
<th>Change in concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>albumin</td>
<td>chronic liver disease</td>
<td>↓</td>
</tr>
<tr>
<td>γ-globulins</td>
<td>cirrhosis, especially autoimmune</td>
<td>↑</td>
</tr>
<tr>
<td>α₁-antitrypsin</td>
<td>cirrhosis due to α₁-antitrypsin deficiency</td>
<td>↓</td>
</tr>
<tr>
<td>caeruloplasmin</td>
<td>Wilson’s disease</td>
<td>↓</td>
</tr>
<tr>
<td>α-fetoprotein</td>
<td>primary hepatocellular carcinoma</td>
<td>greatly ↑</td>
</tr>
<tr>
<td>transferrin</td>
<td>haemochromatosis</td>
<td>normal but 100% saturated with iron</td>
</tr>
<tr>
<td>ferritin</td>
<td>haemochromatosis</td>
<td>greatly ↑</td>
</tr>
</tbody>
</table>

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, CCl₄, 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 disease

### Dose-dependent hepatotoxicity
- paracetamol (in overdose)
- tetracyclines (high doses only)
- azathioprine
- methotrexate

### Idiosyncratic hepatotoxicity
- isoniazid
- halothane
- methyldopa
- rifampicin
- dantrolene

### Steatosis
- aspirin
- tetracyclines
- valproate
- amiodarone

### Dose-dependent cholestasis
- methyltestosterone

### Idiosyncratic cholestatic hepatitis
- chlorpromazine
- erythromycin estolate
- chlorpropamide
- tolbutamide
- nitrofurantoin
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

<table>
<thead>
<tr>
<th>Test</th>
<th>Acute hepatitis</th>
<th>Chronic hepatitis</th>
<th>Cirrhosis</th>
<th>Cholestasis</th>
<th>Malignancy and infiltrations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bilirubin</strong></td>
<td>N to ↑↑</td>
<td>N to ↑</td>
<td>N to ↑</td>
<td>↑ to ↑↑↑</td>
<td>N</td>
</tr>
<tr>
<td><strong>Aminotransferases</strong></td>
<td>↑↑↑↑</td>
<td>↑</td>
<td>N to ↑</td>
<td>N to ↑</td>
<td>N to ↑</td>
</tr>
<tr>
<td><strong>Alkaline phosphatase</strong></td>
<td>N to ↑</td>
<td>N&lt;sup&gt;b&lt;/sup&gt;</td>
<td>N to ↑↑</td>
<td>↑↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>N</td>
<td>N to ↓</td>
<td>N to ↓</td>
<td>N</td>
<td>N to ↓</td>
</tr>
<tr>
<td><strong>γ-Globulins</strong></td>
<td>N</td>
<td>↑</td>
<td>↑</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td><strong>Prothrombin time</strong></td>
<td>N to ↑&lt;sup&gt;a&lt;/sup&gt;</td>
<td>N to ↑</td>
<td>N to ↑&lt;sup&gt;a&lt;/sup&gt;</td>
<td>N to ↑&lt;sup&gt;c&lt;/sup&gt;</td>
<td>N</td>
</tr>
</tbody>
</table>

<sup>a</sup> Marshall & Bangert: Clinical Chemistry, 6th Edition. Copyright © 2008 by Mosby, an imprint of Elsevier, Ltd. All rights reserved.
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

Dr. Nabeel Nuaimi
Department of Pharmacy
Alnoor University College
( Lectures in Clinical Chemistry)
### Functions of plasma proteins

<table>
<thead>
<tr>
<th>Function</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>transport</td>
<td>thyroxine-binding globulin (thyroid hormones)</td>
</tr>
<tr>
<td></td>
<td>apolipoproteins (cholesterol, triglyceride)</td>
</tr>
<tr>
<td></td>
<td>transferrin (iron)</td>
</tr>
<tr>
<td>humoral immunity</td>
<td>immunoglobulins</td>
</tr>
<tr>
<td>maintenance of oncotic pressure</td>
<td>all proteins, particularly albumin</td>
</tr>
<tr>
<td>enzymes</td>
<td>renin</td>
</tr>
<tr>
<td></td>
<td>coagulation factors</td>
</tr>
<tr>
<td></td>
<td>complement proteins</td>
</tr>
<tr>
<td>protease inhibitors</td>
<td>$\alpha_1$-antitrypsin (acts on proteases)</td>
</tr>
<tr>
<td>buffering</td>
<td>all proteins</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Increase</th>
<th>Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypergammaglobulinaemia</td>
<td>malnutrition and malabsorption</td>
</tr>
<tr>
<td>paraproteinaemia</td>
<td>liver disease</td>
</tr>
<tr>
<td>↑ protein synthesis</td>
<td>humoral immunodeficiency</td>
</tr>
<tr>
<td>artefactual</td>
<td>over-hydration</td>
</tr>
<tr>
<td>haemoconcentration</td>
<td>increased capillary permeability</td>
</tr>
<tr>
<td>due to stasis of blood</td>
<td></td>
</tr>
<tr>
<td>during venepuncture</td>
<td>▼ volume of distribution</td>
</tr>
<tr>
<td>dehydration</td>
<td>protein-losing states</td>
</tr>
<tr>
<td>▼ volume of distribution</td>
<td>catabolic states</td>
</tr>
<tr>
<td>▼ volume of distribution</td>
<td>▼ excretion/catabolism</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Class</th>
<th>Protein</th>
<th>Approximate mean serum concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>prealbumin</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>albumin</td>
<td>40</td>
</tr>
<tr>
<td>α₁-globulin</td>
<td>α₁-antitrypsin</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>α₁-acid glycoprotein</td>
<td>1.0</td>
</tr>
<tr>
<td>α₂-globulin</td>
<td>haptoglobins</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>α₂-macroglobulin</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td>caeruloplasmin</td>
<td>0.35</td>
</tr>
<tr>
<td>β-globulin</td>
<td>transferrin</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>low density lipoprotein</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>complement components (C3)</td>
<td>1.0</td>
</tr>
<tr>
<td>γ-globulins</td>
<td>IgG</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>IgA</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>IgM</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>IgD</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>IgE</td>
<td>trace</td>
</tr>
</tbody>
</table>

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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

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Pathogenesis of oedema in hypoalbuminaemia

- Interstitial space
  - Decreasing hydrostatic pressure
- Blood flow
  - Increasing oncotic pressure
- Capillary wall
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
\( \alpha_2 \)- Macroglobulin

- Its plasma conc. is increased in nephrotic syndrome

- It is also an inhibitor of proteases (like \( \alpha_1 \)-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-transporting 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

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

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.
<table>
<thead>
<tr>
<th>Physiological</th>
</tr>
</thead>
<tbody>
<tr>
<td>pregnancy (last trimester)</td>
</tr>
<tr>
<td>childhood</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pathological</th>
</tr>
</thead>
<tbody>
<tr>
<td>often $&gt;5 \times \text{ULN}$</td>
</tr>
<tr>
<td>Paget’s disease of bone</td>
</tr>
<tr>
<td>osteomalacia, rickets</td>
</tr>
<tr>
<td>cholestasis (intra- and extrahepatic)</td>
</tr>
<tr>
<td>cirrhosis</td>
</tr>
<tr>
<td>usually $&lt;5 \times \text{ULN}$</td>
</tr>
<tr>
<td>bone tumours (primary and secondary)</td>
</tr>
<tr>
<td>renal bone disease</td>
</tr>
<tr>
<td>primary hyperparathyroidism with bone involvement</td>
</tr>
<tr>
<td>healing fractures</td>
</tr>
<tr>
<td>osteomyelitis</td>
</tr>
<tr>
<td>hepatic space-occupying lesions (tumour, abscess)</td>
</tr>
<tr>
<td>infiltrative hepatic disease</td>
</tr>
<tr>
<td>hepatitis</td>
</tr>
<tr>
<td>inflammatory bowel disease</td>
</tr>
</tbody>
</table>
Aminotransferases

Two aminotransferases are used in diagnosis and management:

*AST
*ALT
<table>
<thead>
<tr>
<th>Causes of an increased plasma aspartate aminotransferase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Often &gt;10 x ULN</strong></td>
</tr>
<tr>
<td>acute hepatitis and liver necrosis</td>
</tr>
<tr>
<td>major crush injuries</td>
</tr>
<tr>
<td>severe tissue hypoxaemia</td>
</tr>
<tr>
<td>(levels may sometimes exceed 100 x ULN in these conditions)</td>
</tr>
<tr>
<td><strong>5-10 x ULN</strong></td>
</tr>
<tr>
<td>myocardial infarction</td>
</tr>
<tr>
<td>following surgery or trauma</td>
</tr>
<tr>
<td>skeletal muscle disease</td>
</tr>
<tr>
<td>cholestasis</td>
</tr>
<tr>
<td>chronic hepatitis</td>
</tr>
<tr>
<td><strong>Usually &lt;5 x ULN</strong></td>
</tr>
<tr>
<td>physiological (neonates)</td>
</tr>
<tr>
<td>other liver diseases</td>
</tr>
<tr>
<td>pancreatitis</td>
</tr>
<tr>
<td>haemolysis (<em>in vivo and in vitro</em>)</td>
</tr>
</tbody>
</table>

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γ-Glutamyltransferase (GGT)

- an enzyme involved in the transfer of the γ-glutamyl residue from γ-glutamyl peptides to amino acids, H2O, 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 increases in pancreatitis

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

Dr. Nabeel Nuaimi
Lectures in Clinical Chemistry
Department of Pharmacy
Alnoor University College
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

- $17\beta$ estradiol ($E_2$) is the major estrogen of the ovary
  - Ovaries secrete 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**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>day 0: 0900 h; take blood for testosterone; give 5000 U hCG i.m.</td>
<td>normal response: plasma testosterone level increases to above upper limit of reference range</td>
</tr>
<tr>
<td>day 4: 0900 h; take blood for testosterone</td>
<td>primary testicular failure: little or no response secondary testicular failure: response may be normal</td>
</tr>
</tbody>
</table>

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

• breast 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, E₂, 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 breast 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 be 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: 
  \((E_2, \text{estrone and estriol})\)

• 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

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