BODY FLUIDS

Cerebrospinal Fluid

It is a clear colorless bodily fluid found in the brain and spine. It is produced in thechoroid plexus of the brain.

The brain produces roughly 500 mL of cerebrospinal fluid per day. This fluid is constantly reabsorbed, so that only 100-160 mL is present at any one time.

CSF pressure, as measured by lumbar puncture (LP), is 10-18 cmH_2O

It acts as a cushion or buffer for the brain's cortex. It provides a basic mechanical and immunological protection to the brain inside the skull.

The CSF occupies the subarachnoid space (the space between the arachnoid mater and the pia mater) and the ventricular system around and inside the brain and spinal cord.

It constitutes the content of the ventricles,cisterns, and sulci of the brain, as well as the central canal of the spinal cord.

Functions :

1. **Protection:**

   CSF protects the brain tissue from injury when jolted or hit. In certain situations such as auto accidents or sportsinjuries, the CSF cannot protect the brain from forced contact with the skull case, causing hemorrhaging, brain damage, and sometimes death.[12]

2. **Chemical stability:**

   it allows for homeostatic regulation of the distribution of neuroendocrine factors, to which slight changes may lead to problems or damage to the nervous system.

   For example:

   high glycine concentration disrupts temperature and blood pressure control, and high CSF pH causes dizziness andsyncope.

3. **Prevention of brain ischemia:**

   it is made by regulating the amount of CSF in the limited space inside the skull.

4. **Clearing waste:**

it plays an important role in flushing metabolic toxins or waste from the brain's tissues' cellular interstitial fluid (ISF). These findings indicate that CSF may play a large role during sleep in clearing metabolic waste.

5. **Certain biomarkers are increased in Alzheimer disease:**

CSF amyloid beta, total CSF tau protein and P-Tau.

URINE REPORT

A-NORMAL CONSTITUENTS OF URINE

PHYSICAL PROPFRTIES OF URINE

I. Color:

Normal urine is amber yellow in color due to the presence of many pigments including: urochrome, urobilin and traces of uro-erythrin.

The color varies from pale to dark yellow according to urine volume.

Variations of urine color under pathological conditions:

Abnormal Colors Causes

-Nearly colorless - Diluted urine in cases of diabetes insipidus, Diabetes mellitus and chronic nephritis.

- Deep yellow - Concentrated urine as in fevers.

- Reddish - Blood (haemoglobinuria and haematuria).

- Brownish or greenish - Bile pigments, in jaundice.

2.Odor:

Normal urine has aromatic or urineferous odor due to the presence of volatile acids.

Abnormal Odors Causes

- Ammoniacal - On standing due to the effect of bacterial urease on urea producing ammonia.

- Acetone (Sweet apple smell) - Due to the presence of acetone in urine as in uncontrolled diabetes mellitus and starvation

- Bad odor - Pus due to urinary tract infection.

3. Appearance:

Normal fresh urine is clear. On standing urine becomes slightly turbid due to precipitation of phosphate salts at alkaline urine.

Pihnlogical turbidity: due to the presence of blood, pus or crystals.

4.Deposits

No deposits are found in normal fresh urine.

Pathological deposits: due to the presence of blood, pus or crystals.

5. Reaction to litmus paper:

Normal urine is slightly acidic (turns blue litmus paper into red). The pH of normal urine varies with the nature of diet. High protein diets produce acidic urine, while high contents of fruits and vegetables result in alkaline urine.

6. Specific Gravity:

The normal specific gravity of urine ranges between 1015-1025.

Abnormalities Causes:Low specific gravity (1002) - Dilute urine after excessive drinking of water or in case of diabetes insipidus

High specific gravity (1040) occurs in cases of concentrated urine, after excessive sweating or dehydration.

- In diabetes mellitus due to the presence of glucose.

Chemical composition of Urine

A. Normal constituents of urine

a. Organic acids:

- Oxalic acid
- Lactic acid.
- Citric acid
- hippuric acid:

b.Hormonai metabolites:

- Mainly steroid hormones conjugated with sulfate and glucuronate.

C. Non-Protein Nitrogenous Compounds (NPN):

• Total NPN Compounds 25 - 35 g/day.

They include:

1.Amino acids 100-200 mg/day

2. Ammonia 0.7 g/day

3. Urea 25 g/day

4.Uric acid 100-500 mglday

5.Creatine 50-150 mg/day

6.Creatinine 1.4 g\day

1.Ammonia:

It is formed in the renal tubules by glutaminase enzyme that converts glutaihine to glutamate and NH3 (60 %).

The remainder (40%) are formed by deainination of amino acids.

Ammonia excretion is increased in acidosis as in case of ketosis, ingestion of acid forming

Ammonia excretion is decreased in all cases of alkalosis

2.Amino acids:

Amino acids are excreted in urine either free or combined with compounds e.g. hippuric acid is detoxication product of benzoic acid with glycine.

a-Physiological aminoaciduria: Occurs in newly born and in pregnancy.

b-Pathological aminoaciduria: occurs as a result of: Renal aminoaciduria (renal failure) and metabolic errors of amino acid metabolism.

3.Urea:

It is the main end product of protein catabolism.

Urea excretion increases in, cases of increased protein intake and diabetes mellitus, and hyperthyroidisin.

Urea excretion decreases in cases of decreased protein intake, renal failure ,pregnancy and in liver diseases.

4.Uric acid:

It is the end product of purine catabolism.

The average amount of uric acid excreted in urine is 0.1 to 0.5gm \day. It increases in cases of metabolic gout and in malignancies.

5 ,6 Creatine and Creatinine:

Creatine is excreted in very small amounts in urine, since it is completely reabsorbed by the renal tubules.

Creatinine is formed form creatine in constant amounts.

The excretion of creatinine is related to the muscle mass.

It increases in cases of myopathies, after labour and in children.

Creatinine clearance (The amount excreted in urine /minute) is about 90-120 ml/minute.

B. ABNORMAL CONSTITUENTS OF URINE

I .Proteins:

Proteinuria: is the excretion of large amount of proteins (mainly albumin) in urine (more than (300 mg/day).

2 .GIucose

The normal level cannot be detected by fehling or benedict tests. It increases in cases of glucosuria.

3.ketone bodies:

They include acetoacetic acid, beta hydroxy butyric acid and acetone.

4. Bile salts and bile pigments:

Present in cases of biliary obstruction by stones or tumors.

Urinary Deposits

They are classified into:

A- Organised Urinary Sediments:

1-Epithelial cells 2-Red blood cells. 3-Pus cells. 4-Bacteria. 5-Sperms.

6-Casts:

They include the following types:

a-Hyaline casts: Few amounts are present in normal urine. They increase after exercise. They appear as transparent, elongated, refractile tubular fibers.

b-Granular casts: They occur usually in the end stages of acute and subacute nepbritis. They are similar to hyaline casts, but contains dotted granules.

c- **Epithelial blood and leukocyte casts**: They are presence in acute nephritis. They consist of hyaline or granular casts surrounded by adherent cells.

B- Unorganised Urinary Sediments: -

They include:

1-Phosphates:

They are found in alkaline urine as in cases of excessive intae of fruits and vegetables and on standing by the effect of the bacterial enzymes (urease) that hydrolyses urea forming the alkaline ammonia.

2-Calcium Oxalate:

It is frequently found in acidic urine and in cases of excessive intake of tomatoes, mango and other fruits and vegetables rich in oxalate.

The crystals have envelop like shape. They are insoluble in acetic acid.

3.Urates:

- They are present mainly in acidic urine and have different shapes e.g. prisms, needles or rossettes.
- Na, K, Ca, and Mg urates are amorphous and may be pigmented with uroerytbrin.
- Aminonium urates may be deposited from alkaline urine.

4.Calcium carbonate:

They are in the form of spherical masses, not common but may be present in alkaline urine.

URINARY CALCULI (STONES)

Urinary calculi are formed in the urinary tract from urinary sediments. They increase in the lot lowing conditions:

1 In cases of urinary tract infections.

2 In cases of vitamin A deficiency due to roughness of epithelial lining of urinary tract.

3. In cases of metabolic disorders as gout.

Composition:

Urinary calculi

They include the following:

1-Pure calculi: Consists of a single constituent:

- Phosphate stones: soft in consistency with grayish-white color.
- Calcium oxalate: rough with dark brown color.
- Uric acid stones: hard with smooth surface present in cases of hyperuricemia.
- Cystine: greenish in color.

2-Mixed Calculi:

They are composed of more than one constituents.

3-Foreizn body calculi:

Formed of bacteria or products of inflammatory e.g. fibrin and necrosed tissues.

Chemical Detection of Normal Constituents of Urine

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1. Proteins: * Heat coagulation Test

principle of the test:

Albumin undergoes denaturation, by heat followed by flocculation and formation of cross link between precipitated peptides forming a white mass (coagulum)

*procedure: In a test tube add:

*10 ml of the urine.

* Observation: Turbidity appears at the upper part of the tube, indicating the presence of either heat coagulable protein (albumin) or excess phosphates.

2. Glucose :

*Fehling Test:

* Principle of the test:

Free carbonyl group present in glucose can reduce copper ions in alkaline medium giving red ppt.

*procedure: In a test tube add:

-1 ml of Fehling A.

- 1 ml of Fehling B.

-2mL of urine.

- Mix the tube and boil for 2-3 minutes.

* Observation: Glucose gives green, yellow, orange, or red precipitate

3.Ketone bodies: * Rothera s Test:

*procedure. In a test tube add:

-2 ml of the urine

- Saturate it with ammonium sulphate crystals by vigorous shaking.

- Add 2 drops of sodium nitroprusside solution and mix.

- Add 2 ml. of strong ammonia solution. Mix and allow to stand for 10 mm.

* Observation: Permanganate (reddish-violet) color develops with acetone.

5.Bile Salts: *Hay’s Sulphur Test:

*principle of the test: Bile salts lowers the surface tension of urine lead to sinking of sulphur powder.

*procedure:

- Fill a test tube completely with urine.
- Gently sprinkle little amount of fine sulphur powder on the top of the urine and observe the result.

* Observation: In normal urine, sulphur powder will float on the surface of urine while in the presence of bile salts it will sink to urine bottom of the tube.

Urine examination

Practical Examination

Urine sample

Physical Examination

Color; Amber yellow

Odor; Urineferous

Reaction; acidic.

Appearance; Clear

Specific gravity; Reading (from the urinometer) + Room temperature -15 / 3

Chemical examination

Test	***observation***	***result***
Fehling(1ml Fehling A+1mlFehling B+2ml urine)+Heat for 2 min	red,orange,yellow	***glucosurea***
	same as before heating (green)	No glucosurea
Heat coagulation(5ml urine + heating of the upper part of the urine for 3 mins)	coagulum *(white mass)*	***proteinurea***
	no coagulum	no proteinurea
Hay's test;6 ml urine and few sulfur powder	sulfur floats	No Bile salts
	sulfur sinks	***Bile salts (Jaundice)***
Rothera test;2 ml urine + ammonium sulfate (saturation)+1 ml ammonia+ 1drop Na Nitropruside	violet color	***Acetone* (ketosis)**
	No violet color	No Acetone No (ketosis)

Glucosurea

It is the *presence* of *detectable amounts of glucose in urine* (***normal renal threshold is 180 mg/dl)***

Causes of glucosurea;

A) Hyperglycemic glucosurea;

1. Diabetes mellitus.
2. Adrenaline glucosurea; due to stress.

3. Alimentary glucosurea; after gastrectomy, due to rapid evacuation of the stomach.

B) Normoglycemic glucosurea;

1. Congenital renal glucosurea due to abnormal lowering of renal threshold.
2. Renal diseases as (Nephritis).
3. Pregnancy.

Proteinurea

It is the presence of proteins in 24 hours urine sample.

Causes of proteinurea are either physiological or pathological.

Physiological proteinurea

1. Increase protein in diet.
2. Postural (Standing for a long time).

Pathological proteinurea

Pre-renal;1)Leukemia 2) Multiple myeloma (characterized by the presence of Bence Jones protein).

Renal;Due to renal diseases as nephritis.

Post-renal;as inflammations in the urinary tract, as cystitis.

Bile acids

Primary bile acids (synthesized in the liver) are ***cholic and deoxycholic***,

Secondary bile acids (synthesized in the intestine***)*** are ***chenodeoxycholic and lithocholic***

bile salts are sodium glycocholate and potassium taurocholate

Importance of bile acids

1. *emulsification of dietary fat*
2. *formation of micelles for absorption of fat*
3. *A mean of excretion of cholesterol*

4. *They prevent cholesterol precipitation and formation of cholesterol stones*
5. *they have choleretic effect(reabsorption from intestine)that help more bile excretion*

Ketosis

Ketosis is a condition characterized by elevated ketone bodies in blood **(Ketonemia)** and ketone bodies in urine **(ketonurea).**

***Ketone bodies** are acetoacetate, β hydroxybutarate and acetone.*

Causes of ketosis;

1. Prolonged fasting.
2. High fat or low carbohydrate diet.
3. Severe uncontrolled diabetes mellitus.
4. Administration of anti insulin hormones.

Ketogenic substances;

High fat or low carbohydrate diet or administration of anti insulin hormones.

Anti ketogenic substances

Carbohydrates ,glycerol, glucogenic amino acids and insulin.

Metabolic changes with ketosis;

1. ***In adipose tissue***; increase in lipolysis and release of free fatty acids.
2. ***In liver***; increase production of ATP from fatty acid oxidation and decrease oxaloacetate.
3. ***In extrahepatic tissue;*** increase rate of ketolysis.
4. ***In brain***, accommodation to ketone bodies metabolism occurs.

Complication of ketosis

1. ***Acidosis*** if not treated leads to academia and death.
2. ***Electrolyte imbalance*** especially loss of ***Potassium***.

Management of ketosis;

1. Intravenous glucose infusion in case of fasting.
2. Intravenous injection of glucose and insulin in case of diabetes.

3. Bicarbonate in case of acidosis.
4. Potassium in case of electrolyte imbalance.

Urine is formed by:

1- Reabsorption: for minerals, water, glucose and amino acids.
2-Secretion for organic acids, hormonal metabolites, non protein nitrogenous compounds, and hydrogen ions.

(BLOOD)

Blood

It consists of blood cells (55% of blood) suspended in plasma (45% of blood)

A-The blood cells: include : RBCs, WBCs and Platelets

I-Red Blood Cells (Erythrocytes):

Composition of RBC's

RBCs contain proteins, glutathione, amino acids, enzymes, glucose and its metabolites and lipids (glycolipids, phospholipids and cholesterol). They also contain inorganic constituents:The erythrocytes contain high concentrations of K, 2+ and phosphate and low concentrations of Na and Mg

Metabolism of Erythrocytes:

1- Glycolysis:

Anaerobic glycolysis with production of lactic acid is the only source of energy to red cells.

2-HMP:

It provides NADPH for reduction of glutathione.

3-Carbonic anhydrase enzyme:

It plays an important role in CO2 transport from tissue to lung and its elimination through lungs.

4-Rhodanese (cyanide sulfur transferase):

It converts cyanide to thiocyanate to detoxif' it.

5-Other enzymes: e.g. peptidases, catalase, phosphatases and choline esterase are also present.

Il-Leukocytes:

White blood cells count ranges 5,000 - 10,000/ uL . Their main function is the immune reactions.

III -Platelets:

The platelet count ranges 150,000- 250,000 / uL .They play an important role in blood clotting.

They contain proteins, phospholipids, (especially cephalins), the different blood clotting factors and high concentration of histamine and serotonin.

B: THE PLASMA:

I. Plasma proteins.:. .

Plasma contains about 9 % solids, mainly in the form of proteins. The total plasma proteins ranges 6.0 - 8.0 g/dl. Albumin concentration ranges 3.5 -5.5g/dl (represents 55% of total protein)

While globulins range: 2.5 - 3.5g/dl (represents about 45 %of total protein). The concentration of Fibrinogen ranges : 0.2 - O.6g/dl. .

Plasma proteins could be separated by the following methods:

1-Salting out:

Plasma proteins are precipitated by using different concentrations of ammonium sulfate as following:

a- Fibrinogen (by 1/5 saturation of anim. Sulfate).

b- Globulins (by 1/2 saturation of amm. Sulfate). .

c- Albumin (by full saturation of amm. Sulfate).. :

2-Electrophoresis:

Six different proteins bands could be identified as follows:

albumin, alpha1, alpha2 and beta globulins, fibrinogèn and lastly gamma globulins.

3-Other methods:

Ultracentrifuge and immunological analysis can reveal other proteins.

II. Plasma non protein nitrogenous compounds (NPN):

The total NPN compounds in plasma ranges 15 to 60 mg /dl which include:

1-Urea: 20 - 40 mg\dl

2- Free amino acid: 3 - 6 mg\dl

3- Uric acid: 3 - 7 mg/dl.

4- Creatine (0.2 - 0.9 mg/dl) and Creatinine (0.8 - 1.2 mg/dl

Creatinine is the metabolic end product of creatine. Creatinine level usually increases in cases of renal diseases due to decreased excretion.

5-Ammonia: 0.65 - 0.1 mg/dl

Plasma important parameters

I- Carbohydrates:

a-Glucose: The main carbohydrate its level ranges 70-105 mg/dL

b-Fructose: 5-10 mg/dl

c-Pentoses: traces 2 - 3 mg/dl

d-Mucopolysaccharides (GAGs) 80—120 mg/dl

II-**Lipids**:

Total lipids:400-700 mg/dl

a-Cholesterol 100-200 mg/dl

h-Phospholipids 150-250 mg/dl

c-Triacylglycerols 50-150 mg/dl

d-FFA 10-30 mgldL

III-Ketone Bodies: 0.1 -1.0 mg/dl

IV-Bile Pigments: up to 0.8-l.0mg/dl

a- Direct bilirubin up to 0.0-0.2 mg/dl

b-Indirect bilirubin up to 0.5-0.9 mg/dl

V- Minerals

-Sodium 136-145 meq/L

-Chloride 96-106 meq/L

-Potassium 3.5-5 meq/L

-Calcium 9-11 mg/dl

-Phosphorus 3-5 mg/dl

-Sulfur 0.5-1.5 mg/dl

-Iron 50-1 50 ug/dl

Spectrophotometer

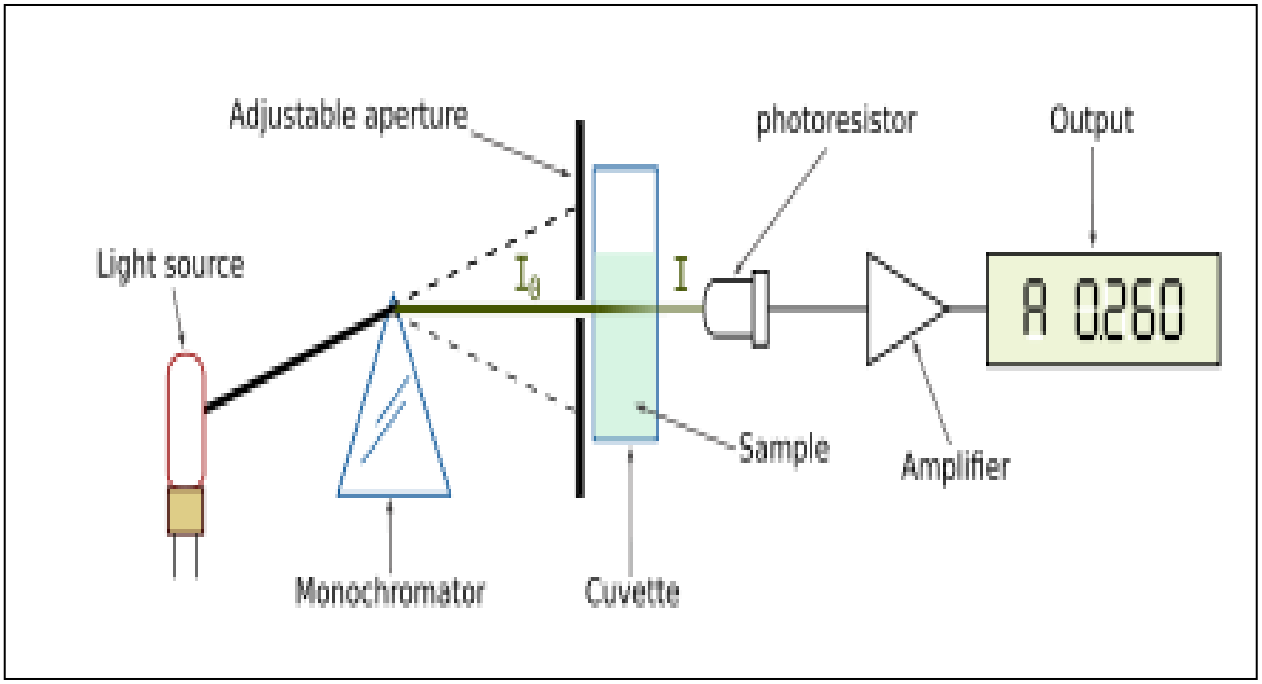
A spectrophotometer is an apparatus used to measure the amount of photons converted into electric current that can measure intensity as a function of the light source wavelength.

Important features of spectrophotometers are spectral bandwidth and linear range of absorption or reflectance measurement.

spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength.

spectrophotometry deals with visible light, near-ultraviolet, and near-infrared.

Principle of spectrophotomerty:



The apparatus is:

The light source is imaged upon the sample.

- ❖ A fraction of the light is transmitted or reflected from the sample.
- ❖ The light from the sample is imaged upon the entrance slit of the mono-chromator.
- ❖ The mono-chromator separates the wavelengths of light and focuses each of them onto the photodetector sequentially.

It is used for measurement of mainly in the routine laboratories as estimation of blood glucose kidney functions and renal functions tests.

Enzymatic Determination of Plasma Glucose

Glucose kit includes:

1-Glucose reagent solution: contains glucose oxidase , peroxidase and phenol

2-glucose standard solution : concentration is 100 mg/ dl.

Principle of the test :

Glucose present in the blood plasma is determined according to the following reaction:

Glucose by glucose oxidase ----> gluconic acid + H_20_2.

Peroxidase

H_20_2 + phenol + 4-amino 4- antipyrine—-------> quinone-imine + 4 H_2O

Method:

Label 3 test tubes: Blank (B). Standard (St.) and Test (T).

	Blank	Standard	Test
-Working solution	1 ml	1 ml	1 ml
Plasma			10 µ1
Standard		10 µ1	
H_20	10 µ1		

Mix, incubate for 10 minutes at 25 °C, read against (B) at 540 nm

Calculation:

Concentration of g1ucos in plasma = $\frac{\text{0.D of the (T) X}}{\text{0.D (St)}}$ Cone of St.= mg/dl.

Determination of Plasma cholesterol

Principle: cholesterol is estimated by the enzyme Cholesterol esterase and gives a product with a depth of color that is directly proportionate with the conc.of cholesterol

The reagent contains:

Cholesterol esterase 125 U/L

Cholesterol oxidase 200 U /L

Standard cholesterol: 200 mg/dl

Method:

Label 3 test tubes: Blank (B), Standard (St.) and Test (T).

	Blank	Standard	Test
-Working solution	1 ml	1 ml	1 ml
Plasma			20 µ1
Standard		20 µ1	
H_20	20 µ1		

Mix, incubate for 10 minutes at room temp, and then read optical density (OD) against (B) at 540 nm..

Calculation:Concentration of cholesterol = $\frac{\text{O.D(T) X Conc. of St.}}{\text{O.D(St)}}$ = mg/dl.

Determination of Uric acid

Principle: by the presence of 2 enzymes uricase and peroxidase uric acid give a red color that is directly proportionate with its conc.

- Standard uric acid: 40 mg/dl

Method:

Label 3 test tubes: Blank (B), Standard (St.) and Test (T).

	Blank	Standard	Test
-Working solution	1 ml	1 ml	1 ml
Plasma			20 µ1
Standard		20 µ1	
H_20	20 µ1		

Mix, incubate for 5 minutes at room temp, and then read against (B) at 540 nm.

Calculation:

$$\text{Concentration of uric acid} = \frac{\text{O.D (T) X Conc. of St.}}{\text{O.D (St)}} = \quad \text{mg/dl.}$$