Physical properties and detection of normal constituents of urine

BCH 472
Urinary System

- The kidneys **remove waste product** from the blood through small filtering units called **nephrons**.

- Each nephron consists of a ball of small blood capillaries, called a **glomerulus**, and a small tube called a **renal tubule**.

- The **kidneys** form urine, which passes through the **ureters** to the **bladder** for storage prior to excretion.

- **Waste product** of protein metabolism are excreted,
  - **electrolyte levels** are controlled
  - and **pH** (acid-base balance) is maintained by excretion of H+ ions.
Urine Formation:
There are three processes involved in the formation of urine:

- Filtration.
- Selective reabsorption.
- Secretion.
1- Filtration:

- This takes place through the **semipermeable** wall of glomerulus and glomerular capsule.

- Water and **small** molecules move from the glomerulus to the inside of the glomerular capsule.

- Molecules which have molecular weight **more** than 70,000 Dalton **can not** pass the glomerulus.

- Blood cells, plasma proteins and other large molecules are **too large** to filtrate.

- Inside the glomerular capsule now contains **glomerular filtrate** which is very similar in composition of plasma except of **plasma proteins** and **blood cells**.

- (non-selective filtration occurs).
2- Reabsorption:

- Reabsorption is the movement of water and solutes from the tubule **back into the blood**.

- as molecules and ions are passively and actively reabsorbed from the nephron into the blood of the peritubular capillary network.

- Nutrients such as glucose and amino acids return to the peritubular capillaries almost exclusively at the **proximal** convoluted tubule.

- every substance has a **maximum rate of transport**.
3- Secretion:

- Is a second way by which substances are removed from blood and added to the tubular fluid.

- Hydrogen ions (H\(^+\)), creatinine, and drugs such as penicillin are some of the substances moved by active transport from blood into the kidney tubule.

- is a process in which the renal tubule extracts chemicals from the capillary blood and secretes them into the tubular fluid.
In the end, urine contains substances that have undergone glomerular filtration but have not been reabsorbed and substances that have undergone tubular secretion.
## Glomerular filtrate vs Urine

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Daily Excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glomerular Filtrate</td>
</tr>
<tr>
<td>Water</td>
<td>130,000 ml</td>
</tr>
<tr>
<td>Sodium</td>
<td>20,000 mmol</td>
</tr>
<tr>
<td>Albumin</td>
<td>4 g (60 µmol)</td>
</tr>
<tr>
<td>Urea</td>
<td>900 mmol</td>
</tr>
</tbody>
</table>
Composition of Normal Urine

- Water 96%
- Urea 2%
- Uric acid
- Creatinine
- Ammonia
- Sodium
- Potassium 2%
- Chloride
- Phosphate
- Sulphate
- oxalate
Urinalysis

- Urinalysis (UA) simply means analysis of urine, it is a laboratory test done to detect problems with your body that can appear in your urine.
Urinalysis

• **Physical Examination:**
  Volume, Specific gravity, Color, Appearance, odor, pH.

• **Chemical Examination:**
  • **Organic:** Uric acid, Creatinine.
  • **Inorganic:** Chloride, Phosphate, Bicarbonate, Sulphate, Ammonia.
1. Physical Examination:

**Volume:**

The daily output of urine on an average diet and normal fluid intake is between 800-2500 ml with an average of 1500 ml/day.

- There are several Factors will affected on urinary output:
  1. Physiological factors
  2. Pathological factors.

- **Physiological:** depends on the *fluid intake* (which is usually a matter of habit) and on the *loss of fluid* by other routes (primarily sweating which, in absence of fever, depends on physical activity and on the external temperature).
### Pathological:

<table>
<thead>
<tr>
<th>Polyurea</th>
<th>Oligurea</th>
<th>Anurea</th>
</tr>
</thead>
<tbody>
<tr>
<td>• More than 2500 ml/day</td>
<td>• Below 500 ml/day</td>
<td>• 100 ml /day</td>
</tr>
<tr>
<td>• Diabetes mellitus</td>
<td>• Incase of deficient intake of water or excessive loss of fluids by</td>
<td>• Stones or tumors in the urinary tract creating an obstruction to</td>
</tr>
<tr>
<td></td>
<td>other routs like hemorrhage or as diarrhea and vomiting</td>
<td>urinary flow</td>
</tr>
</tbody>
</table>
Color:

- Normally, Urine is **clear** and **amber** (yellow) in color due to the presence of **urobilin**

- the **higher** the concentration of urine, the **deeper is the color**.

- Pale urine has a **low** specific gravity, a **dark** line has a **high** specific gravity.

- The concentration of urine is **highest** in the a morning specimen (overnight urine) and is lowest in a specimen passed an hour after much fluid has been taken.

- Colored urines occur in certain **diseases** or metabolic disorders, and after the administration of many drugs.
Odor :

- Normally Urine smells aromatic due to the presence of volatile organic acids.

- The urine of patients with diabetes mellitus may have a fruity (acetone) odor because of ketosis.

- Urine which is infected with Gram-negative organisms often has a distinctive unpleasant smell.

Appearance :

- This is classified as clear and turbid.

- Normal urine is clear.

- cloudy urine causes of turbidity include the presence of blood cells, yeast, and bacteria.
**pH:**
On a normal mixed diet the urine is usually **acid**, generally varying in pH between 5.5 and 8.0, with a mean of 6 in 24 hours.

- **Acidic Urine:**
  - Diabetic ketosis, fevers.

- **Alkaline Urine:**
  - A **vegetarian diet** which causes a tendency to alkalosis.
  - It may also be grossly increased by **bacterial infection** of the urinary tract.

**Specific Gravity:**
- SG is a measure of the density of the dissolved chemicals in the specimen.

- There are **direct relationship** between concentration of substance in urine (Concentration of urine) and SG.

- The normal specific gravity (correctly called relative density) of a pooled 24 hour urine sample is between 1.025 and 1.010.
2. Chemical Examination:

- A series of chemical tests is run. Usually, a chemically impregnated dipstick can be used for many of these tests.

- These urinalysis test strips (dip sticks) have small test patches impregnated with various chemicals in order to detect the presence or absence of certain substances. Qualitative and/or quantitative results can be obtained depending on the particular test.
Test strips (dipsticks)

- The test strips consist of absorbent microfiber cellulose pads attached to it.
- Each pad contains the dried reagents needed for a specific test that react with the compounds present in urine producing a characteristic color.
- There are strips which serve different purposes, such as qualitative strips that only determine if the sample is positive or negative, or there are semi-quantitative.
  
  semi-quantitative strips provide an estimation of a quantitative result, the color reactions are approximately proportional to the concentration of the substance being tested for in the sample.

- The reading of the results is carried out by comparing the pad colors with a color scale provided by the manufacturer.
Practical Part

Experiments

1. Physical properties of Normal urine

2. Chemical Examination of normal Urine (organic and inorganic)
Objectives:

• Simple examination of Urine.
• To detect some of the normal organic constituents of urine.
• To detect some of the normal inorganic ions present in urine.
How to test your urine (visual read)?

A. Prepare some fresh urine sample.
B. Dip the dry strip into the urine.
C. Absorb the excess urine with absorbent paper.
D. Contrast color chart, close to which color?
1- Physical properties of Normal urine:

Results:

<table>
<thead>
<tr>
<th>Test</th>
<th>result</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hour urine volume</td>
<td>ml</td>
<td>800-2000 ml</td>
</tr>
<tr>
<td>Color</td>
<td></td>
<td>Pale yellow (amber color)</td>
</tr>
<tr>
<td>Appearance</td>
<td></td>
<td>Clear</td>
</tr>
<tr>
<td>Odour</td>
<td></td>
<td>Urine-like (aromatic)</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>5.5-8.0</td>
</tr>
<tr>
<td>Specific gravity</td>
<td></td>
<td>1.010-1.025</td>
</tr>
</tbody>
</table>
2. Chemical Examination of normal Urine (organic):

- **Uric acid:**
  - To 2 ml of urine add 1 ml of Bendect reagent, then heated in a boiling water bath for three minutes. *white precipitate* indicates the presence of uric acid.

- **Creatinine:**
  - To about 5 ml of urine add a few drops of a saturated solution of picric acid. On rendering the solution alkaline with a few drops of 10% sodium hydroxide solution, a deep *red color or orange* due to creatinine picrate appears.
3. Chemical Examination of normal Urine (inorganic):

- **Chloride:**
  - 5 ml of Urine + 5 drops of 2N nitric acid + 2N silver nitrate solution.
  - A white precipitate of silver chloride is formed.
  - Silver chloride is precipitated in the presence of nitric acid and silver nitrate.

- **Phosphate:**
  - 5 ml of urine + 5 ml nitric acid + 4 ml of sodium molybdate ---- heat.
  - A yellow crystalline precipitate of ammonium phospho-molybdate appears.

- **Bicarbonate:**
  - 4 drops of concentrate hydrochloric + 5 ml of urine.
  - A slight effervescence occurs due to CO₂ evolution.

- **Sulphate:**
  - Acidify 10 ml of urine with 1 ml dilute hydrochloric acid + 4 drops of 5% barium chloride solution.
  - A white precipitate sulphate is precepitated as of barium sulphate is formed.

- **Ammonia:**
  - 1 ml of 10% sodium hydroxide solution + 5 ml or urine. Boil.
  - The evolved ammonia may be detected by turning moist red litmus paper blue.
<table>
<thead>
<tr>
<th>Test For</th>
<th>reagent</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>saturated solution of picric acid in alkaline condition</td>
<td>Red-orange color</td>
</tr>
<tr>
<td>Uric acid</td>
<td>Bendect reagent after heating</td>
<td>White precipitate</td>
</tr>
<tr>
<td>Chloride</td>
<td>nitric acid and silver nitrate</td>
<td>White precipitate</td>
</tr>
<tr>
<td>Phosphate</td>
<td>concentrated nitric acid and saturated ammonium molybdate</td>
<td>Yellow precipitate</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>concentrate hydrochloric acid</td>
<td>gaseous carbon dioxide.</td>
</tr>
<tr>
<td>Sulphate</td>
<td>dilute hydrochloric acid + 1 ml drops of 5% barium chloride solution</td>
<td>white precipitate</td>
</tr>
<tr>
<td>Ammonia</td>
<td>sodium hydroxide</td>
<td>ammonia gas with sodium hydroxide. This is an alkaline gas. It turns red litmus paper blue</td>
</tr>
</tbody>
</table>
Questions:

• Albumin is not normally detected in any appreciable quantity in urine. Why not?

• What 2 symptoms are seen in a patient developing diabetes mellitus?

• What is the simplest and quickest method of detecting the presence of blood in urine?