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Practical Textbook of BIOCHEMISTRY for Medical Students

As per the Competency-based Medical Education Curriculum (NMC)



DM Vasudevan
Subir Kumar Das

4th Edition

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4 Normal Constituents of Urine

BI11.3 : Describe the chemical components of normal urine.

INTRODUCTION

Normal urine is pale yellow in color. When the output of urine is low, it appears deep yellow. Freshly void urine is clear and transparent. On standing, it may become turbid due to the precipitation of phosphate. Normal urine contains both inorganic and organic constituents. The inorganic constituents include Na^+, K^+, Ca^{2+}, Mg^{2+}, NH_4^+, Cl^-, $H_2PO_4^-$, HPO_4^{2-}, SO_4^{2-}, and traces of HCO_3^-. Normal organic nitrogenous constituents are urea, uric acid, and creatinine. The total nonprotein nitrogen varies 10–15 g/day, depending mainly on the protein intake. In addition, detoxified products like indican and ethereal sulfates are found in the urine.

About 3–5 g of sodium and 1–3 g of potassium are excreted per day. The reabsorption of sodium by the renal tubules is under adrenal cortical control. The chief inorganic constituent of urine is chloride.

The daily output of calcium in urine is about 0.1–0.3 g/day and that of magnesium 0.1–0.2 g/day. The phosphates in the urine are mainly derived from the phosphorous of phosphoproteins, nucleoproteins and phospholipids. It may also come from inorganic phosphates in the diet. Phosphates exist in two forms: (i) alkaline phosphates—phosphates of sodium, potassium and ammonium; (ii) earthy phosphates—phosphates of calcium and magnesium. Excess calcium or phosphates may be excreted in some decalcifying diseases of the bone, such as osteomalacia and hyperparathyroidism.

The sulfates in the urine are derived from sulfur containing amino acids. Inorganic sulfates of the diet are not absorbed in appreciable amounts. Inorganic sulfur in urine is present in oxidized (75–95%) and unoxidized (5–25%) forms. Whereas ethereal sulfate in urine include phenyl sulfate, cresyl sulfate, indoxyl sulfate, skatoxyl sulfate, and indican (potassium indoxyl sulfate). The ethereal sulfate constitutes normally 10% of the oxidized form of sulfates in urine. The total output of ethereal sulfates is ordinarily between 0.04 and 0.1 g/day.

The most significant pigments in urine are urochrome and urobilinogen. Urochrome is a compound of urobilin and urobilinogen. The color of urine is due to urochrome.

Urea is the chief nitrogenous constituent of urine. It constitutes about 90% of the total nitrogen in urine. It is the end product of protein metabolism. Uric acid in urine represents the end product of purine metabolism in the body. Hippuric acid is formed as a result of the detoxification of benzoic acid by conjugation with glycine in the liver and kidneys. Benzoic acid may be derived from foods, preservatives or from drugs, or may be formed in the intestines from bacterial action on phenylalanine and tyrosine.

EXPERIMENTS

Fresh normal urine

I. Physical Character

Experiment	*Observation*	*Inference*
Appearance	Clear	
Volume	Measure and enter	
Color	Amber yellow	
Odor	Aromatic smell	
Reaction to litmus	Blue litmus turns red	Acidic
Specific gravity		

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Determination of Specific Gravity of Urine

This is done with a urinometer. Urinometer (a hydrometer) consists of a thin stem graduated at 60°F (15°C) from 1,000–1,060 corresponding to specific gravities of 1.00–1.06 values. Take sufficient urine in the urine glass supplied with the urinometer. Allow the urinometer to float in urine without touching the sides. Observe the reading at the meniscus and note the temperature of the urine.

Suppose the meniscus of the urine coincides with the reading, 1.01 and temperature of urine is 36°C. Since the urine is at a higher temperature compared to calibration temperature, a temperature correction has to be applied as follows: for every 3°C rise above the temperature of calibration, a correction factor of one is added to the last digit of the observed reading. The difference between 36 and 15 is 21°C, which when divided by 3 gives 7. Thus the corrected specific gravity is: 1.010 + 0.007 = 1.017.

Normal volume of urine is 1,000–2,000 mL/day. The volume is influenced by the intake of fluid and the presence of solutes in it. Excessive perspiration and strenuous exercise decrease the volume of urine. The specific gravity of normal urine usually amounts to 1.012–1.024. But it can be as low as 1.001 when water intake is high, and as high as 1.040 in case of water deprivation. The specific gravity is directly proportional to the concentration of solutes excreted. Normal urine has pH 4.8–7.5 (average pH 6.0). A high protein diet makes the urine more acidic whereas it becomes slightly alkaline on a diet rich in vegetables and fruits.

II. Chemical Tests

A. Inorganic constituents

Experiment	*Observation*	*Inference*
♦ To 3 mL of urine, add 1 mL concentrated HNO_3 and 1 mL silver nitrate solution.	Curdy white precipitate of AgCl	Chloride present
♦ To 5 mL of urine, add 1 mL concentrated HCl and 2 mL $BaCl_2$.	White precipitate of $BaSO_4$	Inorganic sulfate present

(AgCl: silver chloride; $BaSO_4$: barium sulfate; HCl: hydrochloric acid; HNO_3: nitric acid)

- ♦ HNO_3 prevents the precipitation of urates by $AgNO_3$.
- ♦ HCl prevents the precipitation of other inorganic salts like phosphates.

Chemical tests for fresh normal urine:

Experiment	*Observation*	*Inference*
1. a. To 10 mL of urine, add 10 mL of Baryta mixture, filter	♦ White precipitate	♦ Phosphates and inorganic sulfates are directly precipitated by reacting with barium salts
b. To the filtrate add 5 mL conc. HCl, boil for 5 min, and cool	♦ White turbidity or faint red color	♦ Ethereal sulfates (organic) are hydrolyzed by concentrated HCl to inorganic sulfates which are precipitated as $BaSO_4$
2. *Tests for Ca^{2+} and PO_4^{3-}* To 10 mL urine add 3 mL strong ammonia solution, boil, filter, dissolve the ppt by pouring 1:5 glacial acetic acid on filter paper	♦ Gelatinous precipitate of $Ca_3(PO_4)_2$ which is redissolved in the acid	♦ $Ca_3(PO_4)_2$ is precipitated in alkaline medium and redissolved in acid medium
a. To one part of the above solution (or undiluted urine) add equal volume of 4% ammonium or potassium oxalate	♦ White precipitate or turbidity	♦ Ca^{2+} is precipitated as calcium oxalate
b. To the second part add 1 mL conc. HNO_3 and 5 mL ammonium molybdate solution, and boil	♦ Canary yellow ppt	♦ PO_4^{3-} is precipitated as ammonium phosphomolybdate
3. *Test for ammonia* To 10 mL urine add 4 drops of phenolphthalein and 5% NaOH drop by drop till a faint pint color indicates slight alkalinity, boil. Dip a filter paper in phenolphthalein solution and hold it to the vapors from the tube	♦ Colorless phenolphthalein on the filter paper turns pink. The color disappears after sometime as NH_3 evaporates	♦ Ammonia present in urine is liberated by heat

[($Ca_3(PO_4)_2$: tricalcium phosphate; HCl: hydrochloric acid; HNO_3: nitric acid; NH_3: ammonia; NaOH: sodium hydroxide]

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- Ethereal sulfates do not give a precipitate on addition of barium chloride, but only after preliminary hydrolysis.
- Do not add too much NaOH because urea of urine also breaks down to liberate ammonia in alkaline medium.

B. Tests for organic constituents

a. *Hypobromite test*

Experiment	*Observation*	*Inference*
To 3 mL of urine add few drops of alkaline hypobromite solution.	Effervescence of N_2	Urea present

$NH_2CONH_2 + 3NaOBr \rightarrow 3NaBr + N_2 + CO_2 + 2H_2O$

$2NaOH + CO_2 \rightarrow Na_2CO_3 + 2H_2O$

The test can also be done by using 1mL 40% NaOH and a few drops of bromine water instead of NaOBr solution. Reagent must have sufficient bromine.

b. *Specific urease test*

Experiment	*Observation*	*Inference*
To 3 mL of urine add three drops of phenolphthalein indicator and a knife point of urease powder (or soyabean meal), mix and keep for 15 min	Urine turns pink due to alkalinity	Urea is hydrolyzed to NH_3 and CO_2, which form $(NH_4)_2CO_3$ to make the solution alkaline. Urease is the enzyme, which acts only on urea

Experiment	*Observation*	*Inference*
1. *Schiff's test:* Moisten a strip of filter paper with 3% ammoniacal $AgNO_3$ solution. Add to it a drop of urine	Brown or black stain of colloidal metallic silver on the filter paper	Uric acid reduces silver nitrate to metallic silver in alkaline medium
2. *Benedict's uric acid test:* To 5 mL of urine add 1–2 g of anhydrous Na_2CO_3 powder and 5–6 drops of Benedict's uric acid reagent	Intense blue color	Uric acid reduces phosphomolybdic acid to blue phosphomolybdous acid

Benedict's uric acid solution and Benedict's reagent (for reducing sugar) are different (*see* Appendix IV, reagents section).

Experiment	*Observation*	*Inference*
1. *Jaffe's test for creatinine:* Mix 5 mL saturated picric acid solution with 5 mL 5% NaOH, and divide into two parts a. To one part add 5 mL urine b. To other part add 5 mL water	Orange red color No red color (control)	Creatinine present in urine
2. *Test for hippuric acid:* To 5 mL urine add a few drops of 5% NaOH and 10% $FeCl_3$ solution drop by drop	Light brown precipitate of ferric hippurate	Hippuric acid present
3. *Test for urobilinogen:* To 5 mL urine add 1 mL Ehrlich reagent, mix, and keep for 5 min	Red precipitate	Urobilinogen reacts with p-methylaminobenzaldehyde of the reagent to give the colored complex

1. Do not add excess NaOH in hippuric acid test, as this will form ferric hydroxide, a buffcolored ppt.
2. On standing, urobilinogen of urine is oxidized by air to urobilin, which does not give the test.

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Study Notes

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