



October University for Modern Sciences and Arts

Faculty of Dentistry

**Biochemistry II
SGS 246**

Lab Manual

Dr. Aisha Eid



The best of British higher education in the best environment

URINE

A. Study of the Physical Properties of Human Urine:

A.1. Color:

The color of normal urine is amber yellow the presence of a number of pigments principally urochrome together with traces of pigments of urobilin and minute traces of uroerythrin. The color may vary from pale yellow to dark yellow according to concentration.

The color of urine undergoes variations under pathological conditions as follows:

Color	Cause
-Amber yellow	-Normal
-Nearly colorless	-Diluted urine, diabetes insipidus or chronic nephritis
-Deep yellow	-Concentrated urine, fevers
-Reddish brown	-Blood (haemoglobinuria and haematuria)
-Brownish or green	-Bile pigments and in certain types of jaundice

A.2. Odor:

Normal urine has a characteristic aromatic odor due to volatile acids. On standing the odor may become strongly ammoniacal due to alkaline fermentation of urea by bacteria to ammonia. Acetone in urine gives a sweet apple-like smell, as in the case of diabetes mellitus. Pus gives an unpleasant putrid odor.

A.3. Appearance:

Normal fresh urine is clear. On standing urine samples develop slight turbidity due to precipitation of phosphates. Pathological turbidity in urine may indicate the presence of blood, pus, urates, etc...

A.4. Aspects:

No deposits can be found in normal fresh urine.

A.5. Reaction:

Test with red blue litmus paper. Normal urine is generally slightly acidic to litmus. The reaction of normal urine varies with the nature of diet. High protein diets produce acidic urine, whereas fruit or vegetables rich diets result in alkaline urine.

A.6. Specific Gravity:

The average specific gravity of urine is 1015-1025, but it may fall to 1002 in very dilute urine as after copious drinking of water or as in diabetes insipidus. In concentrated urine, particularly after excessive perspiration, the specific gravity may rise to 1040. It is also quite high in diabetes mellitus due to the presence, of glucose.

Measurement of Specific Gravity:

Fill a proper cylinder with the sample of urine provided. Remove any froth on the surface of urine by filter paper. Take the temperature of urine by mean of a thermometer. Float the urinometer in the urine, read and record the observed reading. Correct the specific gravity value for temperature higher or lower than 15°C on the basis of one unit specific gravity for each three degrees of temperature. Thus, for every three degrees of temperature above 15°C add one unit to the specific gravity observed and for every three degrees lower than 15°C subtract one unit.

Example:

Observed sp. gr. of urine at 27°C = 1023

$$\text{Corrected sp. gr. at } 15^{\circ}\text{C} = 1023 + \frac{27-15}{3} = 1027$$

B. Normal Constituents of Urine:

B.1 Urea:

To 3ml of urine in a test tube add about 3ml. of alkaline sodium hypobromite. Notice the intense bubbling which occurs due to evolution of N_2 gas.

B.2 Uric Acid:

To 3ml of urine add 1ml of 10% NaOH and 3ml of Folin's reagent (phosphotungestic acid reagent). A blue color appears due to the presence of uric acid.

B.3 Creatinine:

To 3ml of urine add 1ml of saturated solution of picric acid, followed by 1ml of 10%NaOH; a deep red color is produced. If the concentration of Creatinine in urine is low, the color will be orange.

B.4 Chlorides:

To 3ml of urine add a few drops of dilute nitric acid, then 1ml of silver nitrate solution. A white precipitate appears due to the presence of chlorides

B.5. Phosphates:

To 3ml of urine add 3ml of concentrated nitric acid and 3ml of ammonium molybdate solution and boil. A yellow canary precipitate appears due to the presence of phosphates.

B.6. Sulfates:

Present in small amounts which can be detected after concentrating the urine by adding to the sample dilute HCl and barium chloride solution, a white turbidity indicates the presence of sulfates.

C. Abnormal or Pathological Constituents of Urine:

C.1 Proteins (proteinuria):

Normal urine contains traces of proteins which cannot be detected by the ordinary tests. The presence of abnormal detectable amount of heat-coagulable proteins in urine is considered pathological.

C.1.1. Heat coagulation test:

Filter about 10ml of the urine into a test tube. Heat the upper part of the urine directly on the flame until it boils, keeping the lower portion unheated, to be used as control. If turbidity appears, it indicates the presence of either heat coagulable protein (albumin) or excess phosphates.

To distinguish between the two, add a few drops of 2% acetic acid. The phosphate turbidity will disappear, whereas that due to albumin will even become denser and flocculent. The idea of heating the upper part of the urine is to enable one to detect faint turbidity by comparing the boiled top part with the unaltered, urine below. Faint turbidity is best seen clear against a dark background.

C.2. Glucose or Reducing Sugars (Glucosuria):

Normal urine contains small quantities of reducing sugars which are, however, insufficient to be detected by the ordinary Benedict or Fehling tests.

C.2.1 Fehling's test:

Mix 1ml of Fehling A with 1ml of Fehling B. Add 2ml of urine and boil for two minutes. The production of yellow or brownish red precipitate indicates the presence of glucose or any other reducing sugar.

C.2.2 Benedict's test:

To 5 ml. of Benedict's reagent in a test tube, add 8 drops of urine. Boil vigorously for 2 minutes and allow cooling spontaneously. In the presence of reducing sugars a precipitate is formed which may be red, yellow or green in color depending on the amount of sugar.

C.3. Bile Salts (Hay's Sulfur Test):

Fill a test tube almost to the mouth of the tube with urine. Leave the tube in the rack and then gently sprinkle a little finely powdered sulfur on the top of the urine and quickly observe the result. In normal urine particles remain on the surface supported by the tension of the urine air interface. If, however, the urine contains bile salts; the interfacial tension is reduced and the sulfur particles quickly sink through the urine to the bottom.

C.4. Ketone Bodies:

The ketone bodies are found abnormally in urine, in the condition known as ketosis. Ketone bodies include acetone, acetoacetic acid and β -hydroxybutyric acid. One or all of these components may be present. They are all derived ultimately from fat metabolism when the carbohydrate available in the body is insufficient or when carbohydrate oxidation is impaired.

C.4.1 Rothera's Test for Ketone Bodies:

Saturate 5ml of the urine with solid ammonium sulfate by vigorous shaking for two minutes. Then add a few drops of sodium nitroprusside solution and mix. Then make the mixture strongly alkaline by adding 3ml of a strong ammonia solution. Mix and allow it to stand for a while (up to 20 minutes). A characteristic permanganate color develops indicating the presence of acetone or acetoacetic acid. A reddish brown coloration should be disregarded.

Example for how to write a urine report

I. Physical Properties:

1. **Color:** Amber yellow.
2. **Appearance:** Clear.
3. **Aspect:** No deposit.
4. **Odor:** Aromatic.
5. **Reaction:** Acidic.
6. **Specific Gravity:** Reading of urinometer + $\frac{\text{Room temp} - 15}{3}$

II. Abnormal Constituents:

Experiment	Observation	Result
1. Heat coagulation test for protein	No coagulation	No protein
2. Fehling Test for reducing sugars	Red ppt	Reducing sugars are present
3. Rothera's Test for ketone bodies	No permanganate color	No ketone bodies
4. Hay's Sulfur Test. for bile salts	Sulfur particles float on the top	No bile salts

∴ The urine contains reducing sugars

Physical Properties:

1. **Color:**
2. **Appearance:**
3. **Aspect:**
4. **Odor:**
5. **Reaction:**
6. **Specific Gravity:**

Abnormal Constituents

Experiment	Observation	Result

∴ The urine contains

Physical Properties:

1. **Color:**
2. **Appearance:**
3. **Aspect:**
4. **Odor:**
5. **Reaction:**
6. **Specific Gravity:**

Abnormal Constituents

Experiment	Observation	Result

∴ The urine contains

Physical Properties:

1. **Color:**
2. **Appearance:**
3. **Aspect:**
4. **Odor:**
5. **Reaction:**
6. **Specific Gravity:**

Abnormal Constituents

Experiment	Observation	Result

∴ The urine contains

Physical Properties:

1. **Color:**
2. **Appearance:**
3. **Aspect:**
4. **Odor:**
5. **Reaction:**
6. **Specific Gravity:**

Abnormal Constituents

Experiment	Observation	Result

∴ The urine contains

Lactose Intolerance, Favism & Von Gierke's Cases

1- A mother visited the pediatrician complaining that her one- week- old child infant is suffering from abdominal distension and diarrhea. The attacks occurred shortly after breast- feeding.

a-What would the possible cause for such case?

b- How could these attacks be prevented?

c- Can the baby drink milk later on?

Answer: This baby has lactose intolerance. He should be given lactose free milk. He can when he can ingest drugs containing lactase.

2- A two – year old child had his first meal of Egyptian beans. Shortly after the meal he was pale, drowsy and fainted. Hemoglobin was as low as 4.0 gram/dl. Urine color was not appreciably changed, but sclera was yellow.

a- What is the biochemical pathway affected?

b- What is the defective enzyme?

c- What is the biochemical basis for hemolysis?

Answer: HMP pathway. G6PD deficiency. HMP is very important for production of NADPH, which in turn provides reduced glutathione for removal of H_2O_2 and thus protects the cell from the oxidative damage.

G6PD deficiency will lead to decreased NADPH. Thus exposure of RBCs to oxidizing agents in beans produces RBCs lysis and development of anemia.

3- A medical student develops hemolytic attack after taking the anti-malarial drug primaquine. This severe reaction is mostly likely due to:

1- G6PD deficiency.

2- Pyruvate kinase deficiency.

3- Diabetes.

4-Glycogen phosphorylase deficiency.

4. After administration of aspirin, a child developed an attack of jaundice (yellowish discoloration of the skin and sclera). Blood count showed decreased hemoglobin level. Mention:

- The defective enzyme responsible for this metabolic disease.
- The cause of the decreased hemoglobin level.
- The biochemical basis for the above disease.
- Two other causes for the above disease.

5- A three years old child is complaining from fainting attacks during fasting, associated with tremors and cold sweats. Ultrasound examination revealed enlargement of both liver and kidneys. Blood analysis revealed elevated fatty acids and cholesterol.

- 1- What is the name of the disease? (**Von Geirke's disease**)
- 2- What is the defective enzyme? (**G6phosphatase**).
- 3- Explain the cause of fainting attacks. (**hypoglycemia**)

6. While fasting, a six-month-old infant is suffering from attacks of sweating, drowsiness and sometimes fainting. Examination showed mild enlargement of the liver. During the attacks blood glucose level was very low (45 mg/dl).

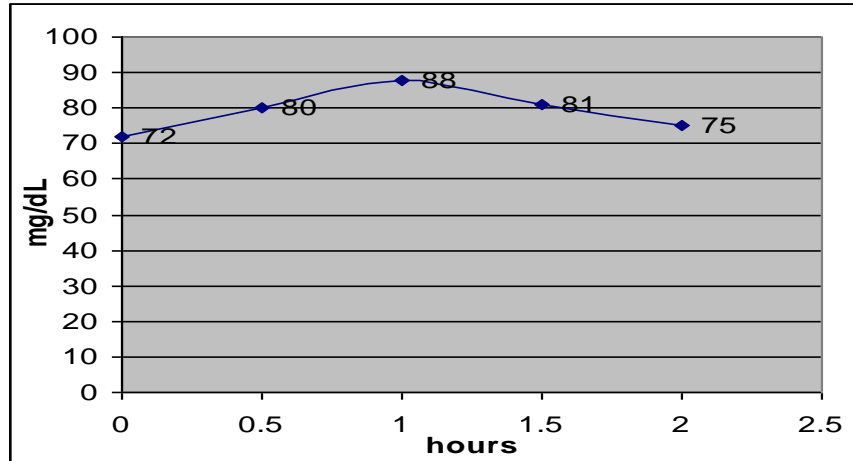
Mention:

- The defective enzyme responsible for this metabolic disease.
- The cause of the enlargement of the liver.
- The other associated metabolic changes in this disease.
- Two other causes for hypoglycemia.

Diabetes

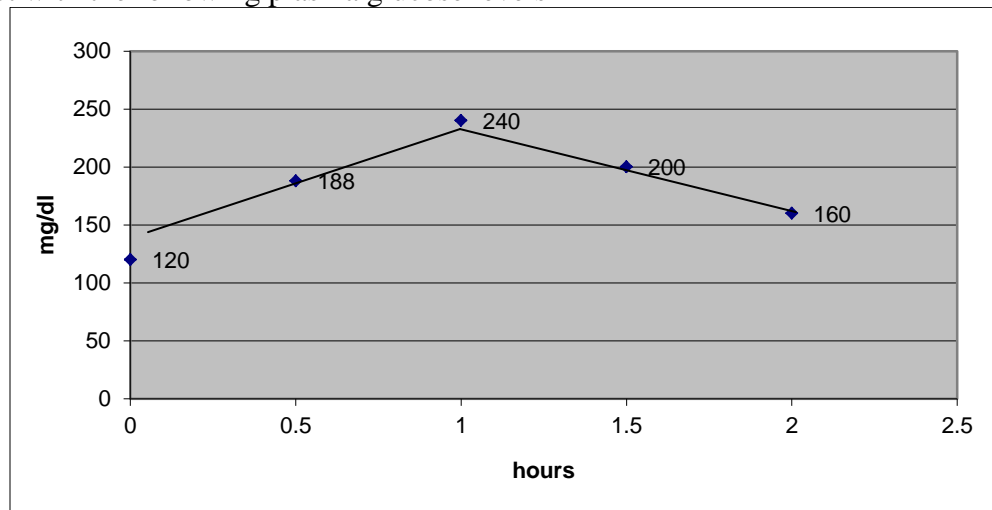
OGTT

I. OGTT of a subject with the following plasma glucose levels



1. The following curve is:
 - A normal response curve
 - A diabetic response curve
2. Do you expect to find glucose in the urine samples taken during this OGTT? Why?
3. Mention the normal fasting plasma glucose level?

II. OGTT of a subject with the following plasma glucose levels



1. The following curve is:
 - A normal response curve
 - A diabetic response curve
2. Mention 2 criteria for your diagnosis?
3. Mention one recommendation for this subject?

Diabetes Cases
Primary Diabetes
Type I (IDDM)

A male subject 15 years old suffering from weight loss, extreme thirst and frequent urination. These symptoms developed quickly over a few days. This subject was diagnosed as diabetic. Mention:

- The most probable type of diabetes.
- The cause of this type.
- The cause of the thirst sensation
- One test to diagnose this condition.
- Treatment of the condition.
- Do you expect that the rise of plasma glucose level was slow or rapid?
- Do you expect that the complications of diabetes to occur more in type 1 or 2 diabetes? Why?

Type II (NIDDM)

1- Overweight 45 year old woman suffering from polyurea. Investigation of urine revealed the presence of glucose. The fasting blood glucose was 200 mg/dl while postprandial sample was 305 mg/dl. Her diagnosis was diabetes mellitus.

- 1- What is the type of diabetes. (Type II)
- 2- What is the type of glucosuria. (Hyperglycemic glucosuria)
- 3- What is the possible cause of this case.(Insulin resistance)
- 4- What is the best diagnostic test for follow up of such cases. (Glycosylated hemoglobin).
- 5- What is the possible treatment. (Reduction of body weight, diet control, oral hypoglycemic drugs).

2.A 45-years old over-weight female complained of polyurea and polydipsia. On plasma glucose analysis her fasting level was 170 mg/dl and 2-hour postprandial level was 290 mg/dL. Comment on the following:

1. Type of diabetes.
2. What is the possible cause for this type of diabetes?
3. What is the test you recommend for follow up of diabetes?
4. Would you expect to find glucose in both fasting and postprandial urine samples? Why?

3.A female subject 50 years complained of feeling more hungry than usual and at the same time was losing weight. This subject was diagnosed as type 2 diabetic. Mention:

- The cause of this type of diabetes.
- The cause for the loss of weight.
- The level of glucose of this subject.
- One test for follow up of the case.
- Do you expect a high incidence of diabetic complications in this subject?

4. An overweight subject 46 years old was diagnosed as type 2 diabetic. Mention:

- The cause of this type of diabetes.
- The level of plasma insulin of this subject.
- One test to diagnose this condition.
- One test to follow up this condition.
- Treatment of the condition.

5. A subject 42 years complained of polyuria. Biochemical investigation revealed that the fasting plasma glucose level was 140 mg/dL and the 2-hour postprandial level was 210 mg/dL. Mention:

- The cause of this polyuria.
- The normal fasting plasma glucose level.
- The level of fasting plasma glucose level diagnostic of diabetes.
- Do you expect to find glucose in the urine of this subject? Why?

Secondary Diabetes **Emotional/Stress Diabetes (Increase anti-insulin hormones)**

1. A male subject 18 years studying for the final examination had a special odour on his breath. The odour was acetone. Urine analysis revealed glucose and acetone. Mention:

- The most probable diagnosis.
- The level of fasting plasma glucose of this subject.
- The level of 2 – hour postprandial plasma glucose of this subject.
- The other symptoms of this subject.
- One test for acetone in the urine.

2. A university student (19 years) complained of polyuria on the days preceding his examination. His doctor advised to perform a test for diagnosis of diabetes.

Comment on the following:

1. Type of diabetes.
2. What is the possible cause for this type of diabetes
3. Treatment of this type of diabetes.
4. Mention one test you recommend for follow up of diabetes.
5. Mention 2-hour postprandial plasma glucose level in normal subjects.

3. A subject was receiving cortisone treatment for a disease of the joints. After few weeks of treatment, this subject became diabetic. Mention:

- The most probable type of diabetes.
- The cause of this type.
- The level of plasma insulin of this subject.
- Treatment of the condition.
- Do you expect to find increased or decreased gluconeogenesis? Why?

Complications of Diabetes

1-A 50 years old, overweight patient presents to your clinic with a wound on her foot. On taking history of her illness, the wound was found to be of 6 weeks duration and a delayed healing power.

The patient also had polyphagia, polydipsia & polyuria.

The laboratory investigations revealed that her fasting blood glucose level was 280 mg/dL, and the serum lipids were elevated.

1- What is the possible diagnosis of this case?

2- What are the suitable tests for diagnosis and follow up of this patient?

3- What are the different complications of the disease?

4- What is the recommended line of treatment?

2. A male subject 18 years was undergoing surgery. The wound of the surgery was healing very slowly and was infected. This subject was diagnosed as type 1 diabetic. Mention:

- The cause of this type of diabetes.
- The cause for the slow healing of the wound.
- The level of plasma insulin of this subject.
- The level of C-peptide of this subject.
- Do you recommend treating the above condition by antibiotics? why

3. A 50-year old over-weight female complained of a wound which was present for a long time without healing. Her doctor diagnosed the condition as diabetes. Comment on the following:

1. Type of diabetes.
2. What is the possible cause for this type of diabetes?
3. Level of insulin in this type of diabetes.
4. Fasting plasma glucose level in diabetic subjects
5. 2-hour postprandial plasma glucose level in diabetic subjects.

4. A patient was complaining of eye disease (retinopathy). His doctor diagnosed the condition as a diabetic complication. Mention:

- The most probable type of diabetes.
- Two other complications of diabetes.
- The normal fasting plasma glucose level.
- The normal 2-hour postprandial plasma glucose level.
- The level of plasma C-peptide of this subject.

Diabetic Coma

1. A ten-year-old boy was admitted to the emergency department to the hospital suffering from coma, rapid, weak pulse, and dry skin. His blood sugar level was 550 mg/dL. His urine contained glucose and acetone. Mention:

- Type of the above diabetes.
- The condition of respiration in the above condition.
- The cause of the dry skin.
- The recommended treatment of the above condition.

2- A two -years old child is admitted to the hospital in deep coma. He was severely dehydrated. His blood glucose was 560 mg/dL; his urine contains both glucose and acetone. His breath revealed presence of acetone odor.

1- What is the type of diabetes? (type I)

2- What is the type of coma (hyperglycemic coma). Describe its different manifestations.

3- Explain the cause of dehydration. (glucosuria causes osmotic diuresis leading to polyuria and hence dehydration).

4- What is the possible treatment? (Insulin and glucose, treatment of associated acidosis, dehydration and electrolytes imbalance)

3. A subject 15 years old was admitted to the hospital in coma. There was acetone odour in his breath. His plasma glucose level was 500 mg/dL. Comment on the following:

- Type of diabetes.
- Condition of the skin and the pulse.

4. On going to work in the morning, a diabetic subject fell comatose. He was sweating heavily. There was no acetone odour in his breath. Comment on the following:

2. His respiration
3. The pulse.
4. Would you expect to find glucose in his urine? Why?

5. On going to work in the morning, a diabetic subject fell comatose. The coma was diagnosed as hypoglycemic coma. Comment on the following:

1. Possible cause of this hypoglycemic coma.
2. Respiration of the subject.
3. Pulse of the subject.
4. Skin of the subject.
5. Would you expect to find glucose in the urine of this subject? Why?

Hypercholesterolemia Cases

1. A male (27 years old), suffered from chest pain and dyspnea. He was advised by his doctor to do some laboratory investigations. His lipid profile showed elevated cholesterol level (750 mg/dL). His doctor prescribed a hypoglycemic drug and advised him to follow a strict dietary regimen.

- a. Mention the possible causes of hypercholesterolemia at his young age.
- b. What are the possible complications of this condition?
- c. What are the important derivatives of cholesterol in our body?
- d. What other investigations do you recommend?

2. A 52 years old female patient suffered from an attack of severe chest pain. Clinical examination of this patient suggested myocardial infarction, and the laboratory investigations revealed hyperlipidemia.

- a. What is the normal range of total blood cholesterol?
How is cholesterol transported in blood?
- b. Mention the different causes of hypercholesterolemia.
- c. Mention the mechanism of atherosclerosis.

3. A 60 years old male patient was suffering from severe chest pain, arrhythmia and dyspnea. He was admitted to the hospital for investigations. Serum cholesterol level was 320 mg/dL and the plasma enzymes used for diagnosis of myocardial infarction showed high activities. The diagnosis was a case of myocardial infarction.

- a. Mention the different plasma enzymes which can help in the diagnosis of myocardial infarction.
- b. Enumerate the different causes of hypercholesterolemia.
- c. What are the important derivatives of cholesterol in our body?

Vitamins

1. A subject who is complaining of malnutrition developed diarrhea, respiratory and gastrointestinal infections. There was xerophthalmia. The condition was diagnosed as vitamin A deficiency. Mention:
 - a. The physiological functions of vitamin A.
 - b. The sources of vitamin A.
 - c. What is xerophthalmia?

2. On examination of a child, there was delayed dentition and delayed closure of the fontanelles. Mention:
 - a. The diagnosis of the condition.
 - b. The deficient vitamin in the above condition.
 - c. Other deficiency manifestations of the vitamin.
 - d. Functions of the vitamin.

Jaundice & Gout

1- A schoolgirl is suffering from fever, anorexia, nausea, vomiting and pain in right hypochondrium. Her stool became pale, clay colored and her urine became dark brown. Her sclera showed yellowish discoloration. Both her direct and indirect bilirubin levels are elevated.

- 1- Which type of jaundice is found in this patient? Why.
- 2- What are the other biochemical tests that could be done?

2- A newborn baby is suffering from severe jaundice. Plasma bilirubin was more than 30 mg/dl. Examination of the family history indicated that his father is Rh positive while his mother is Rh negative. The baby' Rh was positive.

- 1- What is the type of jaundice in this case?
- 2- Which type of bilirubin do you expect to be elevated?

3- A two days old newborn baby had yellowish discoloration of his skin and sclera of the eyes. His parents took him to the doctor for medical investigations.

- 1- What is the possible diagnosis of this case?
- 2- What are the expected laboratory findings?

4- A 45- years old woman suffering from pain in the right hypochondrium radiating to the right shoulder as well as severe itching. Her stool becomes pale in color and her urine is dark brown.

- 1- What is type of jaundice in this case?
- 2- Which type of bilirubin you expect to be elevated?

5- A 35 years old man presented with acute pain in his right big toe. There was no history of trauma or pain in other joints.

- 1- What is the likely diagnosis?
- 2- What are the different diagnostic biochemical tests?
- 3- What is the main drug of choice in this case? How does it act?