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Department of Pathological Analysis Technique.
Subject: - Advanced laboratory techniques.
Lecture-No.4.
General Urine Examination-2.



**The Chemical Examination

To perform the chemical examination, most clinical laboratories use commercially prepared test strips with test pads that have chemicals impregnated into them. The laboratorial dips the strip into urine, chemical reactions change the colors of the pads within seconds to minutes, and the laboratorian determines the result for each test. To reduce timing errors and eliminate variations in color interpretation, automated instruments are frequently used to "read" the results of the test strip.

The degree of color change on a test pad can give an estimate of the amount of substance present. For example, a slight color change in the test pad for protein may indicate a small amount of protein present in the urine whereas a deep color change may indicate a large amount.

The most frequently performed chemical tests using reagent test strips are:

- 1-Specific Gravity (S.G)
- 2-pH
- 3-Protein
- 4-Glucose
- 5-Ketones
- 6-Nitrite
- 7-Bilirubin
- 8-UrobilinogenSpecific

***Gravity (SG): -**

Specific gravity is a measure of urine concentration. This test simply indicates how concentrated the urine is. Specific gravity measurements are a comparison of the amount of substances dissolved in urine as compared to pure water.



***pH: -**

As with specific gravity, there are typical but not "abnormal" pH values. The urine is usually slightly acidic, about pH 6, but can range from 4.5-8. The kidneys play an important role in maintaining the acid-base balance of the body. Therefore, any condition that produces acids or bases in the body, such as acidosis or alkalosis, or the ingestion of acidic or basic foods can directly affect urine pH.

***Protein: -**

The protein test pad provides a rough estimate of the amount of albumin in the urine. Albumin makes up about 60% of the total protein in the blood. Normally, there will be no protein or a small amount of protein in the urine. When urine protein is elevated, a person has a condition **called proteinuria**.

Urine Albumin

Overview

In a healthy renal and urinary tract system, the urine contains no protein or only traces amounts. These consist of albumin (one-third of normal urine protein is albumin) and globulins from the plasma.

Normally, more than 99% of the filtered protein is reabsorbed by pinocytosis in the proximal convoluted tubule Albuminuria

Because albumin is filtered more readily than the globulins, it is usually abundant in pathologic conditions; therefore, the term albuminuria is often used synonymously with proteinuria.

False positive reactions

- False positives occur rarely in highly alkaline urine samples.
- If urine containing residues of detergents,
- Urine of high specific gravity may cause higher than normal readings

***Glucose: -**

Glucose is normally not present in urine. When glucose is present, the condition is called glucosuria. It results from either:

An excessively high glucose level in the blood, such as may be seen with people who have **uncontrolled diabetes**.

A reduction in the "renal threshold;" when blood glucose levels reach a certain concentration, the kidneys begin to eliminate glucose into the urine to decrease blood concentrations.

Some other conditions that can cause glucosuria include: -
hormonal disorders, liver disease, medications, and pregnancy.

When glucosuria occurs, other tests such as a fasting blood glucose are usually performed to further identify the specific cause.

Urine Sugar

Overview:

Glycosuria or **glucosuria** is an abnormal condition of osmotic diuresis due to excretion of glucose by the kidneys into the urine.

The most common cause of glycosuria is **untreated diabetes mellitus** which raises plasma glucose levels far above normal, and beyond a certain threshold, the excess glucose is excreted by the kidneys, taking water with it and producing diuresis.

The threshold varies somewhat from one individual to another, with values around (160 - 180 mg/dl).

False positive reactions The presence of hydrogen peroxide, bacterial peroxidases (e.g. cystitis), will produce false positive reactions.

Drugs: **Nalidixic acid**, **cephalosporins**, **Chloramphenicol**, **Isoniazid** and **penicillin** Stress, excitement, testing after a heavy meal, and testing soon after the administration of intravenous glucose may all cause false-positive results, most frequently trace reactions.

False negative reactions High concentrations of ascorbic acid (>25 mg/dl) inhibit the reaction. Drugs: salicylates, tetracyclines. False-negative results may occur if urine is left to sit at room temperature for an extended period. High specific gravity depresses color development.

Interpretation:

Glucosuria is the excretion of glucose in the urine which may occurs: when plasma glucose concentration exceeds its renal tubular absorptive capacity [renal glucose threshold: (180 mg/d)].

Hyperglycemia without glucosuria occurs when the renal threshold for glucose is raised.

There may be glucosuria without hyperglycemia as in low renal threshold for glucose or renal tubular defects.

***Ketones:** -

Ketones are not normally found in the urine. They are intermediate products of fat metabolism. They are produced when glucose is not available to the body's cells as an energy source.

They can form when a person does not eat enough carbohydrates (for example, in cases of fasting, starvation, or high-protein diets) or when a

person's body cannot use carbohydrates properly. When carbohydrates are not available, the body metabolizes fat instead to get the energy it needs to keep functioning. Strenuous exercise, exposure to cold, frequent, prolonged vomiting, and several digestive system diseases can also increase fat metabolism, resulting in ketonuria. **In a person who has diabetes, ketones in urine may also be an early indication of insufficient insulin.** With insufficient insulin, a diabetic cannot process glucose and instead metabolizes fat.

Urine ketone

Overview:

Ketone bodies: - are three water-soluble compounds that are produced as by-products when fatty acids are broken down for energy in the liver and kidney. They are used as a source of energy in the heart and brain. In the brain, they are a vital source of energy during fasting. The three ketone bodies are acetone, acetoacetic acid, and beta-hydroxyl butyric acid.

ketonemia (Ketosis): is a state characterized by elevated levels of ketone bodies in the blood, occurring when the liver converts fat into fatty acids and ketone bodies (which can be used by all of the body for energy as an alternative to glucose).

Ketonuria: is a medical condition in which ketone bodies are present in the urine. Ketones will be present in the urine when the ketones in the blood go above a certain level.

What is the indication for urine ketone test?

Screening for ketonuria in pregnant women

During pregnancy, the early detection of ketones is essential because ketoacidosis is a prominent factor that contributes to intrauterine death.

Screening for ketonuria in persons with diabetes

1. Testing for ketones is indicated in any patient showing elevated urine and blood sugars (blood sugar levels of **300 mg/dl** or higher)
2. When treatment is being switched from insulin to oral hypoglycemic agents, the development of ketonuria within 24 hours after withdrawal of insulin usually indicates a poor response to the oral hypoglycemic agents.
3. Ketone testing is done to differentiate between diabetic coma positive ketones and insulin shock negative ketones.

Q-) What is the most common Metabolic disease?

- 1-Diabetes mellitus (diabetic acidosis)
- 2-Hyperthyroidism
- 3-Dietary conditions
- 4-Starvation, fasting

5-High-fat diets

6-Prolonged vomiting, diarrhea (cause dehydration)

Q-) What is the main causes of Increased metabolic states?

1-Increased metabolic states caused by:

2-prolonged Fever

3-Pregnancy or lactation

4-strenuous exercise

5-severe stress

6-during acute illness (Approximately 15% of hospitalized patients have ketones in their urine even though they do not have diabetes)

7-Post-surgical condition (Ketonuria occurs after anesthesia (ether or chloroform))

***Nitrite: -**

This test detects nitrite and is based upon the fact that many bacteria can convert nitrate (a normal substance in urine) to nitrite. Normally, the urinary tract and urine are free of bacteria and nitrite. **When bacteria enter the urinary tract, they can cause a urinary tract infection.** A positive nitrite test result can indicate a UTI.

***Bilirubin: -**

This test screens for bilirubin in the urine. Bilirubin is not present in the urine of normal, healthy individuals. **It is a waste product that is produced by the liver from the hemoglobin of RBCs that are broken down and removed from circulation.** It becomes a component of bile, a fluid that is released into the intestines to aid in food digestion.

In certain liver diseases, such as **biliary obstruction** or **hepatitis**, excess bilirubin can build up in the blood and is eliminated in urine. The presence of bilirubin in urine is **an early indicator of liver disease and can occur before clinical symptoms such as jaundice develop.**

***Interfering Factors**

High concentrations of ascorbic acid cause decreased sensitivity.

Aged urine samples

Causes of jaundice: -

Jaundice is classified into three categories, depending on which part of the physiological mechanism the pathology affects.

The three categories are:

1-Pre-hepatic: The pathology is occurring prior the liver

2-Hepatic: The pathology is located within the liver

3-Post-Hepatic: The pathology is located after the conjugation of bilirubin in the liver

***Urobilinogen**

This test screens for urobilinogen in the urine. The results are considered along with those for urine bilirubin (above).

Urobilinogen is normally present in urine in low concentrations. It is formed in the intestine from bilirubin, and a portion of it is absorbed back into the blood.

What is the main indicative of Positive test results?

Positive test results may indicate liver diseases such as viral hepatitis, cirrhosis, liver damage due to drugs or toxic substances, or conditions associated with increased RBC destruction (hemolytic anemia).

When urine urobilinogen is low or absent in a person with urine bilirubin and/or signs of liver dysfunction, it can indicate the presence of hepatic or biliary obstruction.

Urine Urobilinogen

Overview:

***Urobilinogen** is a colorless product of bilirubin reduction. It is formed in the intestines by bacterial action.

Some urobilinogen is reabsorbed, taken up into the circulation and excreted by the kidney. This constitutes the normal "intrahepatic urobilinogen cycle". So normally urine have trace amounts of urobilinogen.



Low or absence of urobilinogen

May be caused by

- Post-hepatic Jaundice
- Impaired intestinal absorption (i.e., diarrhea)
- During broad-spectrum antibiotic therapy, suppression of normal gut flora may prevent the breakdown of bilirubin to urobilinogen; therefore, urine levels will be decreased or absent.