Rothera & Hay's Test

Biochemistry
Third year student

Ketone bodies

Ketone bodies are water-soluble molecules produced by the liver from fatty acids during periods of low glucose availability, such as fasting, prolonged exercise, or low-carbohydrate diets. They serve as an alternative energy source for tissues, particularly the brain, when glucose levels are insufficient.

Let's know each one...

Acetoacetate (2%)

Acetoacetate is the first ketone body produced in the liver. It can be converted into two other ketone bodies: beta-hydroxybutyrate and acetone.

Beta-hydroxybutyrate (20%)

Beta-hydroxybutyrate is the most abundant ketone body in the bloodstream during ketosis. It is transported to peripheral tissues, where it can be converted back into acetoacetate and then further metabolized for energy.

Acetone (78%)

Acetone is a volatile ketone body that is produced as a byproduct of acetoacetate metabolism. It is primarily excreted through the breath and urine.

Ketonuria

- Under normal conditions, metabolized fats are completely broken down to water and carbon dioxide, hence negligible amount (1mg/24 hrs) of ketone bodies are excreted in urine.

- When the rate of production exceeds, excess ketone bodies are eliminated in urine and the condition is known as ketonuria.

- Two conditions most commonly associated are starvation and Diabetes mellitus. Ketonuria is also seen in case of prolonged vomiting, severe diarrhea, anesthesia, liver damage, high fat intake and low carbohydrate intake.

Rothera test

Rothera test is a chemical test used to detect the presence of ketone bodies, specifically acetoacetate, in urine.

Requirements

- Urine specimen
- Test tubes
- **Rothera's powder**:
  Sodium nitroprusside = 0.75 gm
  Ammonium sulphate = 20gm
  Mix and pulverize.
- Liquor ammonia (Ammonium hydroxide)

Procedure of Rothera’s Test

- Transfer about 5 ml of urine to a test tube.
- Add 1 gm of Rothera’s powder mixture and mix well.
- Layer over the urine 1-2 ml of concentrated ammonium hydroxide.
- Observe the pink-purple ring at the interface.

Principle of Rothera’s Test

Acetoacetic acid and acetone react with alkaline solution of sodium nitroprusside to form a purple colored complex. This method can detect above 1-5 mg/dl of acetoacetic acid and 10-20 mg/dl of acetone. Beta-hydroxybutyrate is not detected.

Interpretation:

• The development of a purple color indicates a positive test for the presence of acetoacetate in the urine.

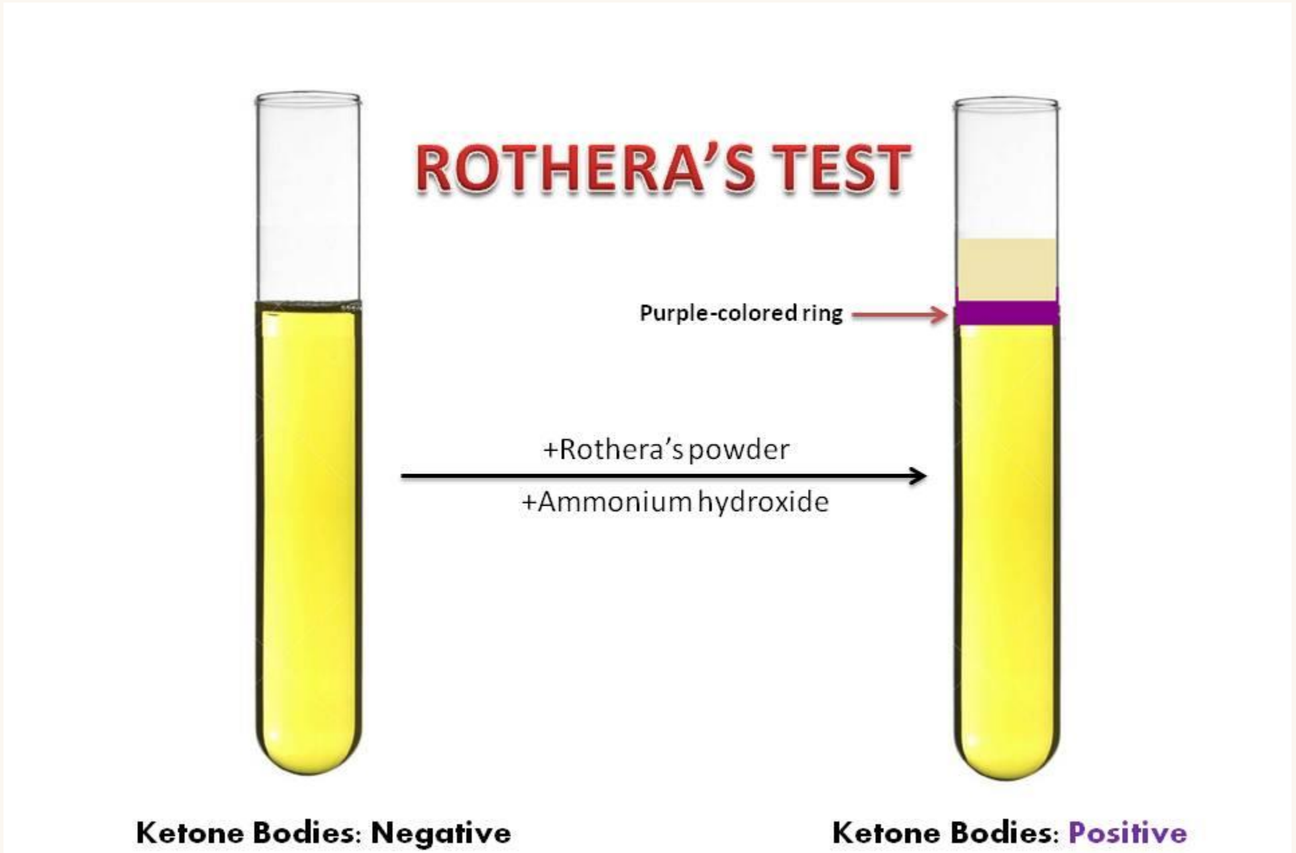
• If no color change occurs, the test is negative for acetoacetate.

Considerations:

• The Rothera test is a qualitative test, providing a yes/no answer regarding the presence of acetoacetate.

• It is relatively sensitive to acetoacetate but may not detect other ketone bodies, such as beta-hydroxybutyrate, which is the predominant ketone body in the bloodstream during ketosis.

• Like other qualitative tests, false-positive or false-negative results may occur, so confirmatory testing may be needed in certain situations.


ROTHERA'S TEST
Purple-colored ring
+Rothera's powder
+Ammonium hydroxide
Ketone Bodies: Negative
Ketone Bodies: Positive

Bile Salts

Bile salts are one of the primary components of bile.

Bile salts help with the digestion of fats. They also help the body absorb fat-soluble vitamins, like vitamins A, D, E, and K.

Bile and bile salts are made in the liver and stored in the gallbladder between meals. In addition to bile salts, bile contains cholesterol, water, bile acids, and the pigment bilirubin.

The presence of bile salts in urine can indicate a disruption in normal bile metabolism and excretion. Under normal circumstances, bile salts are primarily reabsorbed in the small intestine and recycled through the enterohepatic circulation, with only a small amount being excreted in the urine.

However, when there is liver or biliary tract dysfunction, bile salts may accumulate in the bloodstream and eventually be excreted in the urine. This can occur in conditions such as liver disease, cholestasis (obstruction of bile flow), biliary tract infections, or bile duct obstructions.

Hay’s test for bile salts

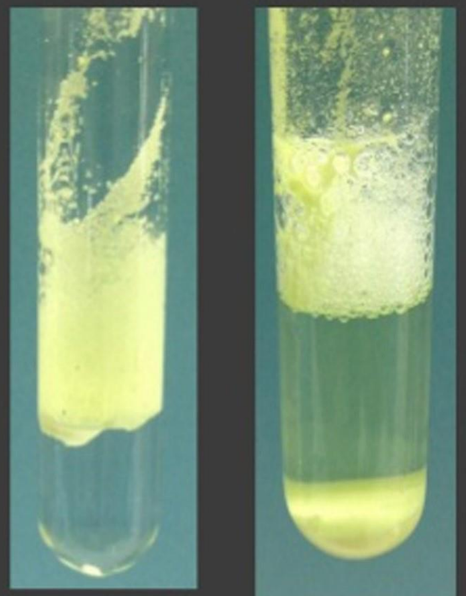
- Hay’s test for bile is a specific test used for the qualitative detection of bile salts in urine. Bile salt appears in the urine of patients suffering from jaundice. It consists of a watery mixture of organic and inorganic compounds.
- also known as Hay's sulphur powder test

Bile salts have a property of lowering the surface tension of the fluid. If Bile salts present in urine and sulphur powder is added to the urine in the test tube, the sulphur particles will sink. In normal cases it does not sink rather, it floats on the surface of the fluids.

- **Hay’s test reagent:**
- Powdered sulphur.
- **Hay’s test procedure:**
- Take 5 ml of urine in a test tube.
- Sprinkle gently a little of finely powdered sulphur “flower of sulphur” on the surface of the urine.

Observations:

Sulphur will sink down to the bottom of the test tube, indicating the presence of bile salts in urine.



Control for comparison

Positive test

Bilirubin in urine

Bilirubin is a yellowish-orange pigment produced by the breakdown of hemoglobin, the oxygen-carrying protein in red blood cells.

Bilirubin is transported in the bloodstream bound to albumin, a protein produced by the liver. It is insoluble in water and must be conjugated (chemically modified) with glucuronic acid in the liver to form water-soluble conjugated bilirubin. Conjugated bilirubin is then excreted into bile and eventually into the intestines.

In the intestines, bilirubin undergoes further breakdown by bacteria to form urobilinogen, which can be reabsorbed into the bloodstream or eliminated in feces. Some urobilinogen is excreted in urine, giving it a yellow color.

The presence of bilirubin in urine, known as bilirubinuria, can indicate liver or biliary tract dysfunction.

Causes of Bilirubinuria:

- **Liver Dysfunction:** Conditions such as hepatitis, cirrhosis, or liver damage can impair the liver's ability to process bilirubin, leading to its accumulation in the bloodstream and subsequent excretion in urine.

- **Biliary Tract Obstruction:** Obstruction of the bile ducts, either due to gallstones, tumors, or other causes, can prevent bilirubin from reaching the intestines, causing it to accumulate in the bloodstream and appear in urine.

- **Hemolytic Disorders:** Increased breakdown of red blood cells (hemolysis) can result in elevated levels of bilirubin, which may overwhelm the liver's capacity to process it, leading to bilirubinuria.

THANK YOU!