

Exercise 32

Aim: To detect the presence of bile salts in the given sample of urine

Principle: Old and damaged RBCs are removed from the circulation mostly in the spleen and to some extent in the liver by macrophages. Hemoglobin of the RBCs is broken down in the cytoplasm of macrophages. When iron is removed from heme component of hemoglobin, the iron-free portion of heme is converted to biliverdin, a green pigment, and then into bilirubin, a yellow orange pigment. Bilirubin enters the blood and is transported to the liver from spleen. In the liver, bilirubin is secreted by liver cells into bile, which passes into the small intestine and then into the large intestine. Bilirubin is detected in urine in certain pathological conditions only.

Requirement: Test tubes, measuring cylinders (10 ml), funnel, dropping pipette or drop bottle, Lugol's iodine solution, barium chloride solution (10%), Fouchet reagent, sulphur powder, concentrated nitric acid, test tube holder, test tube stand, cotton, filter paper, distilled water.

Preparation of reagents

- (i) Lugol's iodine solution: Dissolve 1g of iodine crystals and 2 g of potassium iodide in 100 mL of distilled water.
- (ii) Fouchet reagent: Dissolve 25 mL of trichloroacetic acid (TCA) in 75 mL of distilled water. Now add 1g of ferric chloride to this solution and mix.
- (iii) 10% Barium chloride solution: Dissolve 10 g of BaCl_2 in 90 ml of distilled water and make up the solution to 100 mL with water.

Procedure

(a) Lugol's Iodine Test

Pour 4 ml of urine sample into a test tube. Add 4 drops of lugol's iodine solution to this tube. Shake the tube well and observe. A faint yellow to brown colour indicates absence of bile pigments while light to dark green colour indicates the presence of bile pigments.

(b) Gmelins Test

Take 5 ml of concentrated nitric acid in a test tube. Add an equal volume of the given urine sample to it slowly along the sides of

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the test tube. Formation of a green, blue, yellow or red ring at the junction of the two solutions indicates the presence of bile pigments.

Procedure

Bile pigments present in the urine react with concentrated nitric acid and induces formation of a coloured ring at the junction between the urine and acid layer.

(c) Fouchets Test

Take 5 mL of the given urine sample. Add 2-5 mL of BaCl_2 to this test tube and mix the two solution. A precipitate will appear. Now filter the mixture. The precipitate containing the bile pigments remains on the filter paper. Add 2 drops of Fouchet reagent to the precipitate on the filter paper. If the precipitate turns green, it shows the presence of bile pigments.

Discussion

The colourless bilirubin is oxidised by the ferric ion of ferric chloride (present in the Fouchet Reagent) to green biliverdin.



Questions

1. Give the names of the pigments found in bile.
2. Which organ of the body produces bile pigments?
3. Which pigment provides colouration to the bile?
4. What are the functions of bile pigments?
5. How are the bile pigments produced?
6. Mention the name of the diseases during which excretion of bile pigments occurs in urine.
7. What are the different tests to detect the presence of bile pigments in urine?