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Diploma in Pharmacy 2nd Year
Biochemistry & Clinical Pathology
Experiment
To determine the creatinine in blood /serum.

Aim:

To determine the creatinine in blood /serum.

Reference :

‘ Dr. Gupta G.D. , Dr. Sharma Shailesh, Kaur Manpreet, “Practical Manual of Biochemistry & Clinical Pathology” Published by Nirali Prakashan, Page no 34 – 38

Materials Required

Picric acid, sodium hydroxide, stock creatinine, standard creatinine, flasks, graduated pipettes and photoelectric colorimeter.

Theory

By employing a photoelectric colorimeter and the modified Folin method, creatinine in blood is assessed Protein free blood filtrate, ie, folin Wu is used. The unknown creatinine found in the filtrate is treated with picric acid to create red-colored creatinine picrate in an alkaline medium. The optical density of this red-coloured creatinine is compared to that of standard solution after being converted by picric acid to creatinine picrate The concentration of creatinine in given blood sample can be calculated using colorimetry principle.

Clinical Significance

1) When creatine phosphate spontaneously degrades in the body, creatinine, the waste product of creatine metabolism, is produced. It is a non-threshold substance. The glomeruli often filter it. Changes in its excretion can be a sign of specific metabolic disorders because its excretion is not related with food protein.
2) Its excretion rises during fevers, starvation, consumption of a diet low in carbohydrates, and in diabetes mellitus.
3) It may rise as a result of excessive tissue damage that releases creatine or as a result of improper phosphorylation of creatine.
4) Therefore, creatinine excretion should be used as a measure of endogenous protein metabolism since it is independent of food proteins.
5) An adult of average size normally has an endogenous creatinine clearance that varies from 100 to 130 ml/minute, which is a rough measure of glomerular filtration rate.
6) Reduced glomerular filtration rate is indicated by values below 90 ml/minute.
7) Normal value is 0.7 to 2.0 mg/100 ml blood.

Procedure

1) **Preparation of Unknown Sample:**
   i) 5 ml of folin wu filtrate should be pipetted out in a flask labeled as "S".
   ii) 2 ml of 1% picric acid should be added and mixed well.
   iii) 10% of 0.5 ml of sodium hydroxide solution should be added.
   iv) Then, the solution should be kept for 15 minutes and the optical density should be measured using green filter (530 Mμ). It should be noted as "Es"

2) **Preparation of Standard Sample:**
   i) 5 ml of standard creatinine solution should be pipetted out in a flask labeled as "S"
   ii) 2 ml of 1% picric acid should be added and mixed well.
   iii) 10% of 0.5 ml of sodium hydroxide solution should be added.
   iv) Then, the solution should be kept for 15 minutes and the optical density should be measured using photoelectric colorimeter with green filter (530 mμ). It should be noted as "Es".

3) **Preparation of Blank Sample:**
   i) 5 ml of distilled water should be pipetted out in a flask labeled as "B"
   ii) 2 ml of 1% picric acid and 0.5 ml of 10% sodium hydroxide solution should be added to it.
   iii) Then, the solution should be kept for 15 minutes and compared in a calorimeter using green filter (530 mμ).

Calculations

The optical density of the blank should be subtracted from the optical density of the unknown and the standard by using the principle of photoelectric calorimetry.

$$\text{Concentration of creatinine in unknown blood sample CU} = \frac{\text{Optical density of (Eu) unknown sample}}{\text{Optical density of standard sample "Es"}} \times \text{Concentration of standard creatinine (Cs)}$$

Standard creatinine solution is prepared by diluting Iml of stock solution (1 mg) to 500 ml.

Here,

1 ml of standard creatinine solution = 0.002 mg creatinine.
5 ml of standard creatinine = 0.01 mg creatinine.
Now, 5 ml filtrate = 0.5 ml blood

Therefore

$$\text{Concentration of creatinine in unknown blood sample CU} = \frac{\text{Optical density of unknown sample "(Eu)"}}{\text{Optical density of standard sample "Es"}} \times \text{0.01 mg creatinine}$$

= “X” mg (suppose)

Now "x" mg of creatinine is present in 5 ml of folin wu filtrate.

But 1 ml of folin wu filtrate = 0.1 ml of blood

5 ml of folin wu filtrate = 0.5 ml of blood

"x" mg of creatinine is present in 0.5 ml of blood.

To estimate milligram percentage of creatinine

If

0.5 ml = "x" mg creatinine

100 ml xx100 0.5 mg creatinine

Substituting the value of 'x'

Then.

$$\text{"cu"} \quad \frac{Eu}{Es} \times \frac{1}{100} \times \frac{100}{1} \times \frac{10}{5} = \frac{Eu}{Es} \times 2\text{mg Es 10 15 x 2mg \% creatinine}$$

$$CU = \frac{Eu}{Es} \times 2\text{mg \% creatinine. Es}$$

Result :

Creatine concentration in blood /serum was determined.