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

Aim:

To determine the glucose 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 39 – 43

Materials Required

Alkaline copper sulphate solution, phosphomolybadic acid, sodium tungstate 10%, sulphuric acid 2/3 n, benzoic acid solution, stock glucose solution, standard glucose solution no. 1, standard glucose solution no. 2, fluoride oxalate solution, folins sugar tube, pepettes graduated (2 ml, 5 ml), flasks and photoelectric calorimeter.

Theory

Folin Wu (modified) method is used in which protein-free filtrate (also known as folin-Wu filtrate) is produced so that 10 ml of the filtrate can make 1 ml of blood sample. By treating the blood proteins with tungstic acid, protein-free filtrate is produced. Then an alkaline copper sulphate solution is used to heat this protein-free filtrate that contains glucose. Hence, glucose breaks down copper sulphate to form an equivalent amount of cuprous oxide.

To form an equivalent amount of molybdenum blue, this cuprous oxide is reduced with phosphomlybdic acid The intensity of the molybdenum blue colour depends on the amount of cuprous oxide, which is related to the amount of glucose present in a particular sample of "folin wu" filtrate

Figure 1: Folins Sugar Tube

Reactions

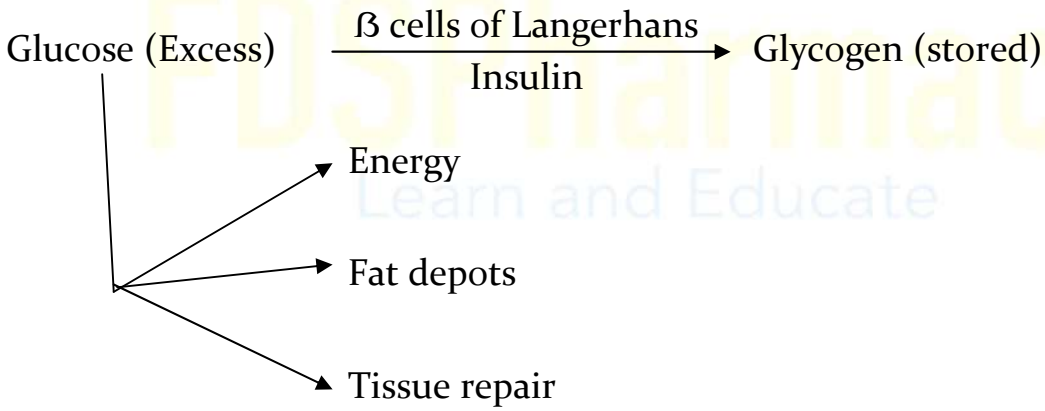
$C_6H_{12}O_6 + CU^{++} \longrightarrow CU^{++}$ Oxidation
Glucose cupric products of glucose

$CU^{+}\ Na_2\ PO_4 : 12\ MoO_3 \longrightarrow CU^{++} + 12\ MoO$
cuprous cupric molubdenum blue

A similar procedure and a photoelectric calorimeter are used to compare the blue colour of the standard solution to the blue colour produced from the test blood sample. The optical density of the test and standard are determined and the colorimetric principle can be used to determine the blood glucose levels.

Clinical Significance

1) Monosaccharides, like glucose, fructose, galactose, etc. are the main end product of carbohydrate digestion absorbed in the blood, and glucose metabolism provides:
   i) Major amount of energy for body activities
   ii) Reserve fat depots
   iii) Tissue glycolipids
   iv) Amino acids
2) Thus, it would seem that the metabolism of glucose plays a crucial part in the metabolism of carbohydrates, which is closely linked to the metabolism of protein and fat.
3) The hormones of the islets of Langerhans control the availability of glucose from various dietary sources and its management and utilisation. Following is a schematic explanation of this:



4) As a result, the blood sugar level is kept stable, normal, and expressed as fasting, ie, after a meal.
5) A real symptom of diabetes mellitus is hyperglycaemia, or an increase in blood sugar levels. Excess free glucose accumulates in the blood as a result of inadequate insulin production or impaired insulin secretory function.

6) According to the severity of the condition, high fasting blood sugar readings in diabetes mellitus range from normal to 500 mg/100 ml and above.
7) Blood sugar levels above 500 mg/100 ml are related with an increased risk of coma.
8) Blood sugar levels are increased by 150 mg% as a result of the thyroid, pituitary and adrenal glands' hyperactivity, which also involves emotional stress states.
9) Convulsions and the latter stages of numerous diseases are both accompanied by a similar rise in blood sugar levels.
10) Hyperglycaemia, a moderate increase in blood sugar levels, can be caused by sepsis and a number of infectious diseases.
11) Additionally, various brain illnesses, such meningitis, encephalitis, tumours, and haemorrhages are associated with an increase in blood sugar levels.

Preparation of Reagents

1) **Sodium Tungstate:** 10% w/v of 10gm of Na_2 WO, $2H_2O$ (sodium tungstate) should be dissolved in 100 ml of distileed water.
2) **Sulphuric Acid:** 3.5gm of 2/3 N of sulphuric acid should be dissolved in 100 ml of distilled water.
3) **Alkaline Copper Sulphate Solution:**
   i) A solution A should be prepared by dissolving 40gms of anhydrous sodium carbonate and 7.5gms of tartaric acid in 400ml of distill water.
   ii) A solution B should be prepared by dissolving 4.5gm of copper sulphate crystalline in 200ml of distill water.
   iii) Solution B should be added to solution A with constant surring and the final volume should be maintained with distilled water to 1 litre.

4) **Phosphomolybdic Acid Reagent:** 70gm of molybdic acid, 10gm of sodium tungstate AR and 400ml of Sodium hydroxide 10% should be dissolved in 400ml of distil water. It should be boiled for 30 minutes to remove ammonia. Phosphoric acid of specific gravity 1.75 should be added. Distilled water should be added to make the final volume of 1 litre.
5) **Benzoic and Solution:** 2.5gms of benzoic acid should be dissolved in 1000 ml (1 litre) of distil water and heated.
6) **Stock Glucose Solution (1gm/100ml):** 1gm of glucose (AR) should be dissolved in 100 of ml benzoic acid solution.
7) **Standard Glucose Solution No. 1 (10 mg/100ml):** 1 ml of stock glucose solution should be dissolved in 100 of ml benzoic acid solution
8) **Standard Glucose Solution No. 2 (20 mg/100 ml):** 2 ml of stock glucose solution should be dissolved in 100 of ml benzoic acid solution.

Procedure

1) Three folin-wu tubes should be cleaned and labelled as:
   i) Unknown 'U'
   ii) Standard I-Std I
   iii) Standard II - Std II
2) 2ml of "Folin wu filtrate" should be taken to the folin wu tube labelled as "U".
3) 1ml of standard sugar solution 1 (0.1mg sugar) should be taken in a folin wu tube labelled as "Std I".
4) 1 ml of standard sugar solution II (0.2mg sugar) should be taken in a folin wu tube labelled as "Std II".
5) 1ml of alkaline copper sulphate solution should be added to all three tubes.
6) Tubes should be kept in boiling water bath for 6 to 8 minutes.
7) Then, the tubes should be removed from the water bath and 1ml phosphommolyddic acid should be added to all the tubes.

8) The tubes should be again kept in boiling water bath for 2 minutes and thes cooled at room temperature after 2 minutes.

9) 25ml of distilled water should be added to each tube and mixed well and recorded. The optical densities should be compared using photoelectr colorimeter having tube filter 420 mμ.

Calculations

By using photoelectric calorimetry principle

$$\text{Concentration of glucose in given blood sample (Cu)} = \frac{\text{Optical density of unknown blood glucose sample Eu}}{\text{Optical density of standard glucose Es}} \times \text{Concentration of std glucose (Cs)}$$

Now as 1 ml of standard sugar solution is used for experiment it is fact that:
1 ml of standard sugar solution = 0.1 mg of sugar.
ie. Cs concentration of standard glucose = 01 mg of sugar.

Therefore,

Concentration of glucose in given blood sample $CU = \frac{Eu}{ES} \times Cs$

$= \frac{Eu}{ES} \times .1mg$ $\frac{Eu}{ES}$ = " x " (suppose)

Now "x" mg of sugar is present in 1 ml of folin wu filtrate of given blood sample, ie, in 0.1 ml blood.

Note: (1 ml o folin wu filtrate = I ml of blood)
To determine mg percentage of sugar

If 0.1 ml blood = "x" mg of sugar
100 ml blood = 100xx mg 0.1 ml blood.

On substituting the value of 'x'
Then,

$$Cu = \frac{100}{0.1} \times \frac{Eu}{ES} \times .1$$

Concentration of unknown glucose in given blood sample = $\frac{Eu}{ES} \times 100$ mg percent sugar.

$$\text{Concentration of glucose in given blood sample (Cu)} = \frac{\text{Optical density of unknown blood glucose sample Eu}}{\text{Optical density of standard sugar solution}} \times 100$$

Result :

Glucose concentration in blood /serum was determined.

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