Chapter-12

TECHNIQUES AND METHODS FOR GLUCOSE ESTIMATION

Salender Singh*
Assistant Professor, Glocal College of Paramedical Science and Research Centre, Glocal University, Saharanpur, U.P., India.

Swarnima Singh
Assistant Professor, Glocal College of Paramedical Science and Research Centre, Glocal University, Saharanpur, U.P., India.

***Correspondence to:** shailendra@glocalmedicalcollege.in*

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ABSTRACT

The vital origin of liveliness in a human body is Glucose. In an organ system, brain cells and red blood cells are almost whole-body dependent on carbohydrates as the energy source. The production of energy from carbohydrates is 4 kcal per gram. The molecular formula of glucose is $C_6H_{12}O_6$ and glucose is sweet in taste. Glucose is the subcategory of carbohydrates and overall, the most abundant monosaccharide, an important fuel for body. When an individual is well fed, the amount of circulating glucose comes mostly from the food eaten and when an individual is there in a fasting condition the two major metabolic cycles- gluconeogenesis and glycogenolysis are accountable for maintaining the glucose concentration.

Keywords: *Carbohydrates, Glucose, Glucose estimation, Monosaccharide, Metabolic Cycles, Gluconeogenesis, Glycogenolysis.*

12.1 INTRODUCTION

Starch and Glycogen are the basic carbohydrates which gets hydrolyzed into smaller amounts by the action of different enzymes. The single sugar unit carbohydrates are absorbed in intestine and out of all monosaccharide, glucose enters into the liver most. There are numerous metabolic pathways in a living system which uses glucose, for example- Hexose Mono phosphate shunt, glycolysis etc. The levels of glucose in the blood stream are totally controlled by the pancreas which balances the level of glucose to avoid any medical condition related to glucose levels like hypo or hyperglycemia.

12.2 PANCREAS

The pancreas is an organ and gland present in the abdominal cavity in the back of stomach and in front of the spine. Glands are the organs that produce and release substances in the body The pancreas performs two main functions: The exocrine and endocrine function *(J Michael M. et al, 1990).*

1. **The exocrine function:** The exocrine function of pancreas is production of pancreatic juice (enzymes), these juices promote the digestion of carbohydrates, proteins and fats.
2. **The endocrine function:** Production of hormones (insulin) and glucagon, these hormones controls and maintains the amount of glucose levels in bloodstream.

12.3 FUNCTIONS OF GLUCAGON

In human body carbohydrate is stored as Glycogen. It is kept in reserve in two major sites and these sites are liver cells and the muscles. During fasting state, the

glycogen reserved in the liver is responsible to supply glucose as a source of energy, glycogen breaks down in glucose in a cycle called glycogenolysis. The amount of glycogen content in liver is 10 gm per 100 gm tissue which is greater when compared with skeletal muscle, which is 1-2 gm per 100gm tissue. But if the total amount of glycogen is to be observed than the exact amount of muscle glycogen is found to be more than liver glycogen because the muscle mass is much larger than the liver. When the quantity of plasma glucose is on lower side, the glycogen stored in liver undergoes glycogenolysis and helps to balance plasma glucose level on the other side muscle glycogen is to conduct as stock up fuel for muscle contraction (Vivek N. A. et al, 1998).

1. **Stimulation of Glycogenolysis by Glucagon:** In starving conditions glucagon increases the production of glucose by glycogen reserved in the liver to producing a rapid rise in the levels of blood glucose and maintain a balance to avoid hypoglycemia.
2. **Stimulation of gluconeogenesis by glucagon:** Glucagon is also responsible for formation of the energy source glucose from pyruvate, glycerol, lactate and amino acids producing a delayed but more towards comfortable rise in the amount of blood glucose levels.
3. Glucagon is also a powerful lipolytic agent, it releases the FFA and glycerol into the stream blood circulation when needed by the body.
4. Glucagon also plays a vital role in ketogenic action, in liver, excess of FFA are transferred to ketone bodies, i.e. acetoacetic acid, acetone and β-hydroxybutyric acid, by the working mechanism of glucagon.

12.4 ACTIONS OF INSULIN

Insulin is an essential hormone released by the pancreas, that helps to balance and manage the blood sugar levels. Insulin has its different mechanism of action on various metabolic cycles. There are some major actions of insulin which are depicted below-

1. **Action through Insulin on Carbohydrate Metabolism:** Action of insulin on carbohydrate metabolism plays a very important and serious role in maintaining healthy levels of blood glucose in body. Glucose arrival flow into different body cells is increased by the action of insulin, however many of the cells of body including brain, kidney tubules, gastrointestinal tract and RBCs does not need the action of insulin for glucose transport.

There are some important working mechanisms through which insulin is also accountable for developing hypoglycemia explained below-

a. Insulin increases the peripheral usage of glucose.

b. It induces breakdown of glucose in muscles, adipose tissue and liver causing excess of glycolysis resulting hypoglycemia.

c. It stimulates the production of glycogen and parallely interferes with the enzymatic activity responsible for glycogen breakdown.

d. Insulin also reduces the glucose output from the liver resulting in hypoglycemia.

2. **Action of on Fat Metabolism:** Insulin is a vital regulator for fat, protein and glucose metabolism. It stimulates the synthesis of free fatty acids and triglycerides in the adipose tissue, muscle and liver. Insulin can increase uptake of the ketone bodies present in the muscle and inhibits lipolysis in the adipose tissue.

3. **Action of insulin on Protein Metabolism:** Insulin stimulates the producing mechanism of amino acids and protein and inhibits the loss of proteins in muscles.

4. **Glucose In Urine (an abnormal constituent):** Glucose in urine is not observed in a normal healthy individual, the existence of glucose in urine is consider as an abnormal constituent because the existence of glucose in urine indicates high plasma glucose concentration.

5. **Reabsorption and Excretion of Glucose through Renal Tubules:** The reabsorption and excretion processes of glucose is being contingent on the function of plasma glucose concentration.

Normal FBS level: 70 – 110 mg/dl

Normal PP S level: 70 – 140 mg/dl

Normal RBS level: 70 – 140 mg/dl

1. When the concentration of the amount of glucose in plasma levels is low at that stage the reassimilation of content of glucose in renal tubules is (100%) complete. However, in this condition glucose is not excreted in urine.

2. When se the concentration of plasma glucose is more than 180 – 200 mg/dl, the reabsorption of glucose is not complete in the renal tubules and it releases out in

urine and the clinical condition is called as glycosuria (Gogoi J. B. et al, 2018). This stage when at which glucose first appears in urine and plasma glucose concentration is increased is called to be as renal threshold for glucose. The value of 200 mg/dl of arterial and 180 mg/dl of venous plasma is the actual/real renal threshold. This is because while passing through tissues 20 mg/dl of glucose concentration gets utilized.

12.5 QUALITATIVE ESTIMATION OF GLUCOSE

Qualitative Estimation of Sugar in Urine by Benedict's Method: Benedict's test is a chemical test that is used to check the existence of reducing sugar in an analyte, it can also be utilized for detecting the existence of glucose in a urine sample.

Principle of Benedict Test: In an alkaline medium when reducing sugar is heated with Benedict's reagent the existence of sodium carbonate converts the sugar into a strong reducing agent called enediols, when sugars are present in analyte, the cupric ions (Cu2+) in Benedict's reagent are reduced to cuprous ions (Cu+). The actual color of Benedict's reagent is sharp blue, it changes to green, yellow, orange or red, according to the concentration of glucose amount present in urine.

Protocol for Reagent: Benedict's Qualitative Reagent

1. 5.0 ml of Benedict's reagent (blue in color) needs to be taken in a test with the help of graduated pipette for accurate measurement.
2. In second step eight-ten drops of urine sample is added in a test tube slowly by using a dropper called as Pasteur pipette.
3. Handle it carefully with a test tube holder, place the test tube on top of the flame on the flame of either a gas burner or a spirit lamp, heat the mixture gently for two minute or boiling water bath can also be used for heating the mixture for five - ten minutes.
4. Then the mixture is going to changes its color depending to the amount of sugar present in a urine sample which is utilized for the test, now allow the mixture to cool using a beaker containing tap water, place the test tube stand there, once it cools down then notice the results. The results for the Benedict's test are depicted in the ***table 12.1.***

Easy blood glucose monitoring at home by GLUCOMETER: Glucometer is a portable electrochemical medical device which is utilized to measure the amount of glucose in your blood by pricking the finger blood drop. **Figure-12.1** explains the proper utilization

of glucometer for blood glucose monitoring. Glucometer kit contains a digital meter operated by a battery, lancets (small needles), a lancet holder that adjusts the depth of the pricking finger depending on skin thickness and test strips.

Table-12.1: Benedict's Test

Observation of color	The concentration of reducing sugar in g% in the sample	Clinical Interpretation
Blue, no change in color	0	No presence of reducing sugar
Green precipitate	0.5 – 1	Presence of traceable amount of reducing sugar
Yellow precipitate	1 – 1.5	Presence of a little amount of reducing sugar
Orange-red precipitate	1.5 – 2	Presence of moderate amount of reducing sugar
Brick-red precipitate	>2	Presence of large amount of reducing sugar

Working Principle of Glucometer: Colorometric method and amperometric method are the two procedures which are utilized in measurement of glucose which are based on electrochemical technology. In colorometric technique photo sensors forms an electrical connection that forwards analog signals to the device which is followed by transimpidence amplifier for measurement of glucose. **Figure-12.2** shows the working mechanism of glucometer. In amperometric based technology, the electrochemical test strips have electrode embedded with required enzymes and reagent. Glucose undergoes chemical reactions in which electrons production takes place. In next step measurement of electrons is done, the range of electrons is proportional to the quantity of glucose present in that blood sample. Glucometer test strips contains enzymes required for glucose estimation which reacts with glucose present in blood sample and an electronic current is develop by the glucometer which runs through blood sample on strip and then reads the concentration of glucose. Enzymatic reaction takes place in test strip and glucometer is the device which acts as detector in glucose estimation. However, there are various factors which affects the working of a glucometer. Blood glucose test strips plays a vital role in estimation of glucose levels through glucometer as all the complicated reactions take place in strips only.

Figure-12.1: Utilization of glucometer for blood glucose monitoring.

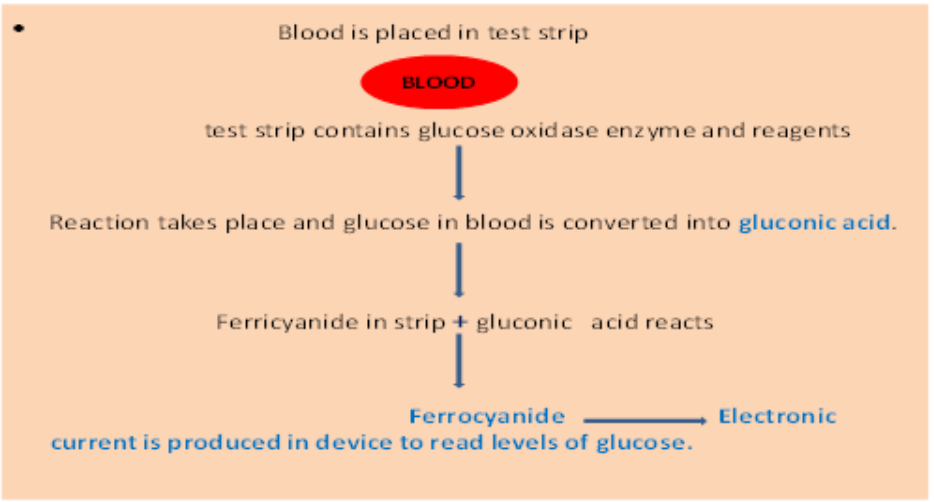

Figure-12.2: Working mechanism of glucometer.

12.6 ENZYMATIC DETERMINATION OF GLUCOSE IN THE LABORATORY

Method

Glucose oxidase-peroxidase (GOD-POD) method is a quick and easily manageable enzymatic reactions colorimetric method which is utilized for the quantitative analysis of D-glucose which exists in the given sample (Gupta P. P. et. al., 2017).

Principle

In the contiguity of atmospheric oxygen, amount of glucose which is present in sample is oxidized by the enzyme glucose oxidase to produce hydrogen peroxide (H_2O_2) and gluconic acid.

$$\text{Glucose} + H_2O + O_2 \xrightarrow{\text{oxidase}} \text{gluconic acid} + H_2O_2$$

The amount of hydrogen peroxide is further break down to water and oxygen by enzyme peroxidase.

$$H_2O_2 \xrightarrow{\text{Peroxidase}} H_2O + O_2$$

The oxygen reacts with 4 – aminophenazone in the existence of phenol to develop a pink color compound and intensity of which could be determined at 550 nm.

Specimen: Specimen used for GOD-POD method is venous blood with sodium fluoride anticoagulant plasma or serum collected within a stipulated time of 30 minutes of blood collection.

Protocol

Take three test tubes & mark them as 'S' (standard), 'T' (test), 'B' (Blank). Now add reagent, sample, standard and distilled water as mentioned in ***the table 12.2.***

Table-12.2: Protocol

REAGENTS	TEST	STANDARD	BLANK
GLUCOSE REAGENT	1.0 ml	1.0 ml	1.0 ml
SAMPLE	0.01 ml	-	-
GLUCOSE STANDARD	-	0.01 ml	-
DISTILLED WATER	-	-	0.01 ml

Table-12.3: Test Parameters

Reaction Type	End point
Wavelength	500 – 550 nm
Flow Cell Temperature	25˚ C
Incubation	15 min at 37˚C
Sample Volume	10 µl/05 µl
Reagent Volume	1000 µl/500 µl
Standard Concentration	100 mg/dl
Blank Zero	distilled water

Now mix the substance in test tubes and follow the process of incubation of 15 min for all three test tubes at 37˚C. Then carefully observe the absorbance of the solution at 550 nm using a colorimeter and at last calculate the results to find out the amount of glucose present in a given specimen. ***All the test parameters are tabulated in Table-12.3.***

12.7 CONCLUSION

Glucose is very much required to regulate various metabolic cycles in a living system. Abnormal levels of glucose can lead to numerous health disorders due to hypoglycemia or hyperglycemia. To avoid the health issues because of abnormal glucose levels or to monitor conditions like diabetes, it is desirable to do continuous monitoring of glucose levels.

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