



Determination of plasma glucose concentration

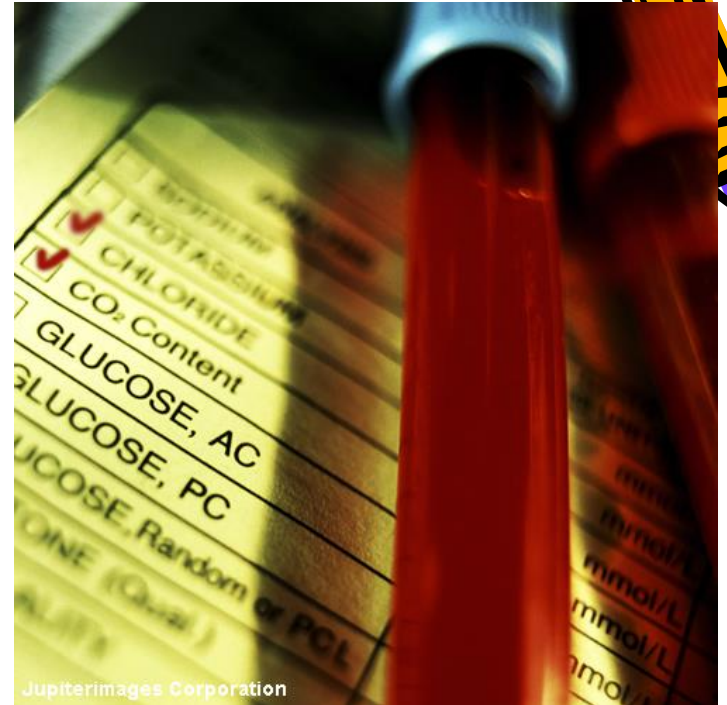


What is blood glucose?

Glucose is a type of sugar used by the body to provide energy.

Sometimes there is too much glucose in the blood, and sometimes there is not enough.

What affects the level of blood glucose?



- **Eating** causes blood glucose levels to **rise**.
- **Vigorous exercise** causes blood glucose levels to **fall**.

How does the body regulate blood glucose levels?



Controlling blood glucose



Between meals, blood glucose levels are topped up from stored deposits in the liver and muscles.

After a meal, blood glucose rises but quickly returns to normal. Where does the excess go? Why not leave it in the blood?

Excess glucose makes the blood plasma and tissue fluid around cells too concentrated. This can severely damage cells, for example, causing **crenation** in red blood cells.



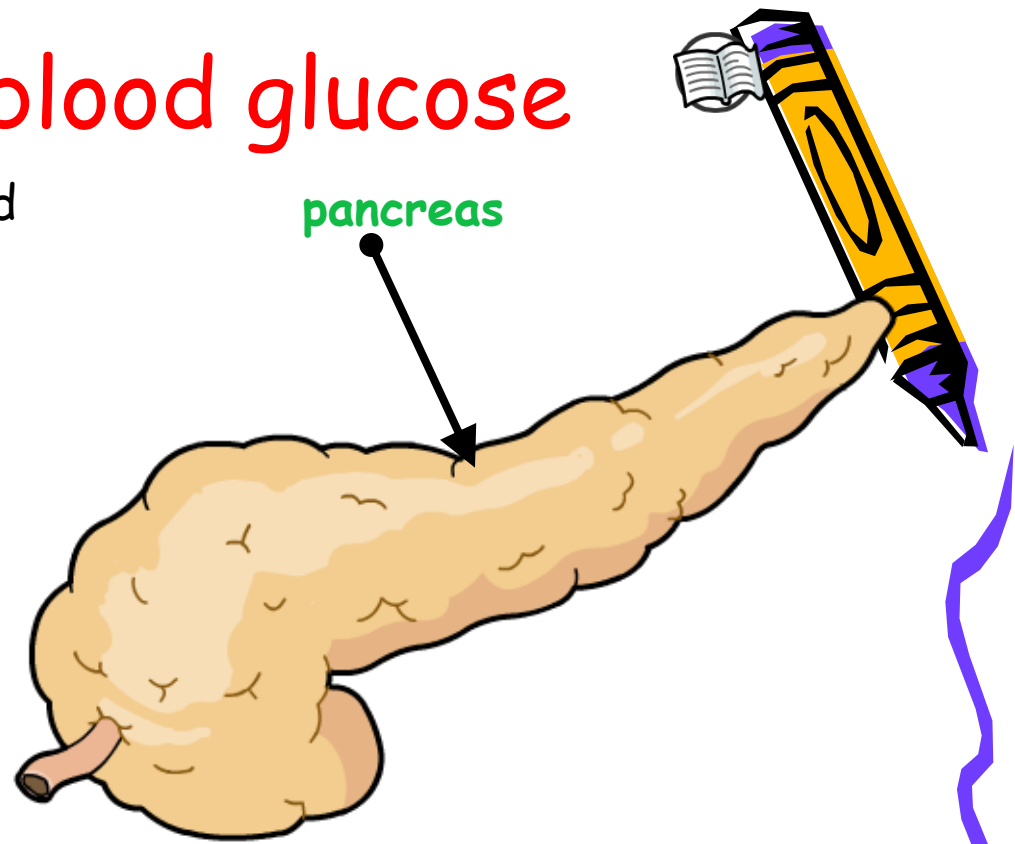
However, low blood sugar levels can be equally as dangerous, as it can make cells swell up and burst. This is called **lysis**.



The pancreas and blood glucose

Blood glucose levels are monitored and controlled by the **pancreas**.

The pancreas produces and releases different hormones depending on the blood glucose level.



Insulin is released when blood glucose levels are **high** – the liver ● stores excess glucose as glycogen.

Glucagon is released when blood glucose levels are **low** – the liver ● converts stored glycogen into glucose and releases it into the blood.



Diabetes mellitus



A group of metabolic disorders that requires continuing medical care and patient-self management education to prevent acute complications and to reduce the risk of long term complications.

It is characterized by hyperglycemia and abnormal protein, fat and carbohydrate metabolism due to defects in insulin secretion, i.e., inadequate and deficient insulin action on target tissues.





It is classified into 4 clinical classes:

- Type I diabetes mellitus (T1DM) •
- Type 2 diabetes mellitus (T2DM) •
- Gestational diabetes mellitus (GDM) •
- Other specific types due to other causes e.g. Drugs or chemical Induced. •



Diabetic Profile Tests:



Group of tests that are used to diagnose diabetes and to measure the treatment response

These Tests include:

-C-peptide, Differentiates between type I and type II. ➤

-Blood Glucose-

-Fasting blood glucose (FBG)

-It also called Fasting Blood Sugar (FBS) = PG=FPG)

-Post prandial glucose (PP)

-GGT= Glucose tolerance test, OGGT= Oral glucose tolerance test

-HbA1c = Glycosylated hemoglobin. ➤

-Ketones. ➤

-Microalbuminuria. ➤

Insulin. ➤

ICA = islet cell antibodies. ➤





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Colorimetric estimation of blood glucose



Medical importance of blood glucose determination

Diagnosis and care of diabetic patients

Assess the effectiveness of proper insulin dose

Blood glucose determination

Reveals individual patterns of blood glucose changes

Allows for quick response to high blood sugar



Blood glucose:

It is a vital component of diabetes management

Types of blood glucose tests

Fasting blood sugar (FBS)

Definition: it measures blood glucose after fasting for at least 8 hours

Sample preparation: fasting at least for 8 hours before the blood sample is taken.

Uses: to diagnose diabetes mellitus when Fasting blood glucose levels significantly elevated ($>140\text{mg/dl}$ or $> 7.77\text{ mmol/l}$)

Conformation Tests:



Glucose Tolerance Test

Comprehensive history, physical examination and other tests

Should be done in other diseases that usually diagnosed with elevated plasma glucose levels, such as overactive thyroid gland and pancreatitis.



Blood glucose:



2-hour postprandial blood sugar (2-hour PP)

Definition: it measures blood glucose exactly 2 hours after eating a meal.

Sample Preparation: For a 2-hour postprandial test, the patient should eat a meal exactly 2 hours before the blood sample is taken.

A home blood sugar test is the most common way to check 2-hour postprandial blood sugar levels.

Random blood sugar (RBS)

Definition: it measures blood glucose regardless of fasting.

Several random measurements may be taken throughout the day because glucose levels in healthy people do not vary widely throughout the day. Blood glucose levels that vary widely may indicate a problem.

Sample preparation: No special preparation is required before having a random blood sugar test.



Blood glucose:



Oral glucose tolerance test (OGTT)

Definition: Oral glucose tolerance test is a series of blood glucose measurements that is used to measure the body's ability to uptake glucose.

This test is commonly used to diagnose diabetes that occurs during pregnancy (gestational diabetes).

This test is not commonly used to diagnose diabetes

Sample preparation: The patient on the test day will be asked to drink a glucose syrup which contained a measured concentration of glucose (75g to 100g) while the pregnant women will take 100g of glucose. samples will be collected at timed intervals of 1, 1.5 2, 2.5 and 3 hours after drinking the glucose.



Diagnostic criteria of diabetes

Fasting blood glucose (FBG)

- *Normal fasting glucose* <100 mg/dl
- *Impaired fasting glucose (IFG)* 100–125 mg/dl

Postprandial blood glucose (2hpp)

- *Normal 2-hours postprandial glucose* <140 mg/dl
- *Impaired glucose tolerance (IGT)*..... 140–199 mg/dl



Aim of practical session

The aim of this practical session is to:

1. estimate blood glucose concentration by glucose oxidase reaction .
2. Perform oral glucose tolerance test.
3. Interpretate the resulting data whether it shows normoglycemic or hyperglycemic or hypoglycemic results.

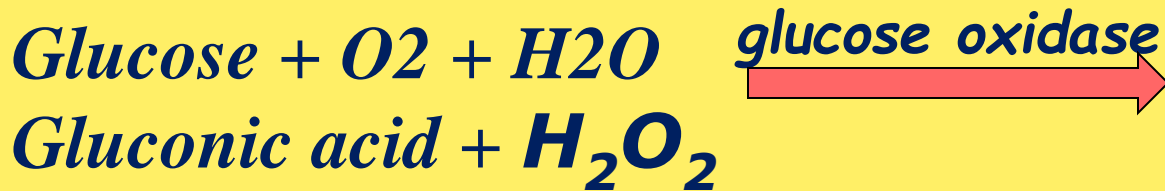


**colorimetric estimation of blood
glucose
by glucose oxidase reaction**



Principle of the glucose oxidase reaction

• Glucose is oxidized by glucose oxidase to form gluconic acid with liberation of hydrogen peroxide.



Principle of the glucose oxidase reaction

- Hydrogen peroxide is dissociated to water and oxygen atom by a peroxidase enzyme.
- The liberated oxygen is captured by a chromogen (a mixture of 4-amino antipyrine & phenol) which is converted to a red violet complex.

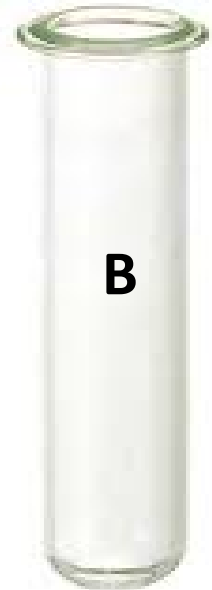


Principle of glucose oxidase reaction

A **colorimeter** can be then used to measure the **intensity** of the color produced; the more glucose present the darker the color.



Step 1: Label 3 dry test tubes



For the test
sample

For the
Standard
sample

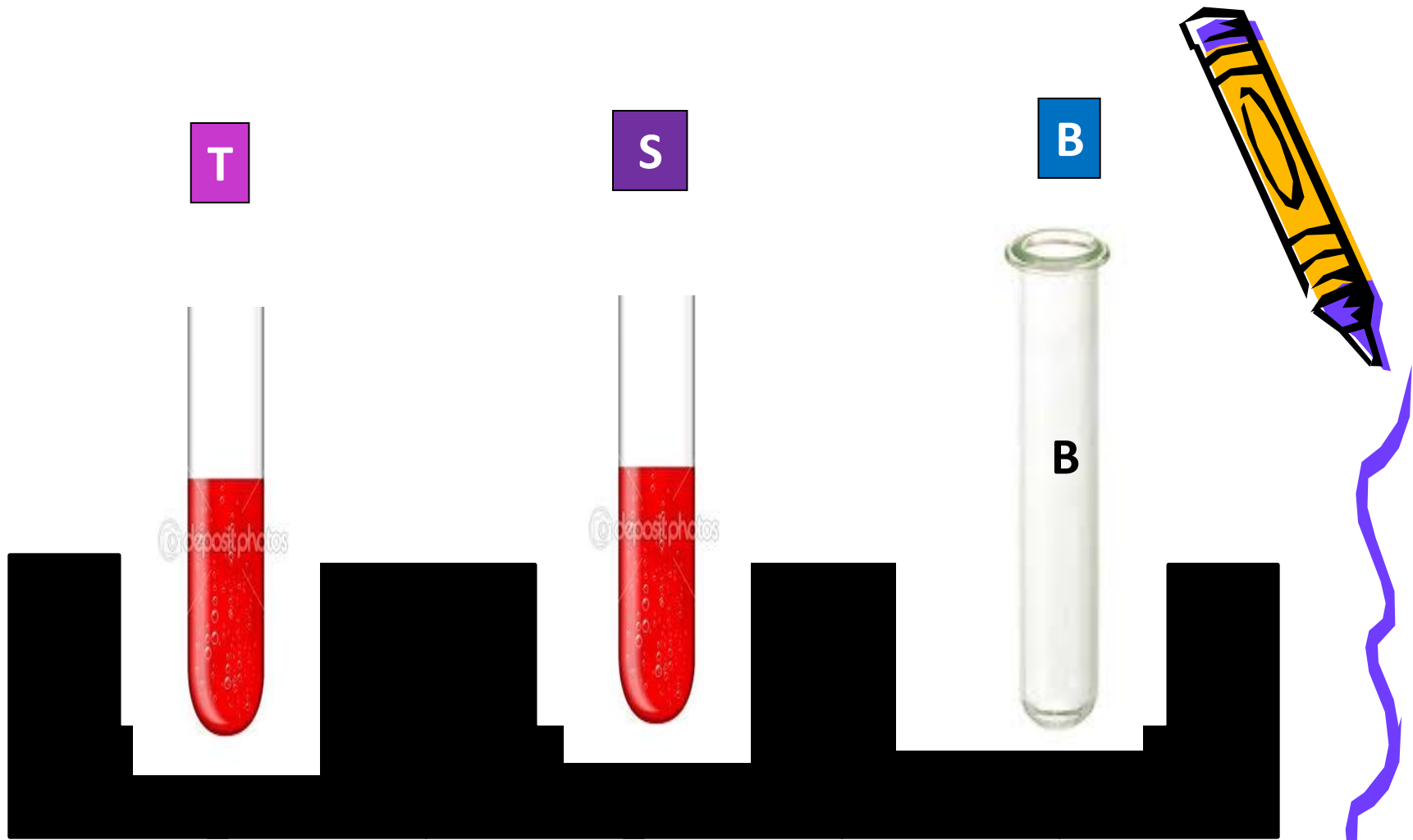
For the
Blank



Step 2: Practical

Determine the glucose concentration in the provided sample as follows:

	Blank	Standard	Sample
Distilled water	10 microliter	—	—
Glucose standard	—	10 microliter	—
Serum/plasma	—	—	10 microliter
glucose reagent	1 ml	1 ml	1 ml



Step 5: Mix the contents of the tubes and incubate 5 minutes 37 C





Step 6: Record the absorbance of both test & standard against the blank at 510 nm.



Calculate the test concentration by the following equation:



Concentration of blood glucose (mg/dl) =

$$\frac{\text{Absorbance of Test}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$



Diabetes Diagnosis

Stage	Test	
	Fasting Plasma Glucose (FPG)	2- Hour Oral Glucose Tolerance Test
Diabetes	≥ 126 mg/dl	≥ 200 mg/dl
Pre-diabetes	≥ 100 and < 126 mg/dl	≥ 140 and < 200 mg/dl
Normal	< 100 mg/dl	< 140 mg/dl

Renal glucosuria



The urine sample corresponding to the peak of the curve contains glucose

The plasma glucose level is still below the normal renal threshold **< 180 mg**



Causes of hyperglycemia

- 1- Diabetes mellitus : Type 1 & type 2.
- 2- High epinephrine : severe emotional stress, pheochromocytoma.
- 3- A rare glucagon secreting tumor.
- 4- High cortisol : Cushing syndrome or high ACTH.
- 5- Intestinal gluosuria.
- 6- Gestational diabetes.

Causes of hypoglycemia

1- Neonatal hypoglycemia:

2- Infantile hypoglycemia:

- a) Glycogen storage diseases in liver.
- b) Impaired gluconeogenesis. (deficiency of glucose 6 phosphatase or fructose 1,6 bisphosphatase).
- c) fructose induced hypoglycemia. (deficiency of aldolase B).
- d) Sever classic galactosemia
- e) Deficient fatty acid oxidation (Carnitine deficiency)



Causes of hypoglycemia



3- Adult hypoglycemia:

- a) Over dose of insulin or oral hypoglycemic drugs.
- b) Insulinoma.
- c) Hypofunction of suprarenal cortex.
- d) Chronic alcoholism.
- e) Post prandial hypoglycemia due to sever over shooting of insulin.



Practice sheet



A male patient estimated his fasting plasma glucose twice (2 weeks between the two assays). The results were 120mg/dl and 122mg/dl. This patient was diagnosed as:

- 1) Diabetic
- 2) Pre diabetic
- 3) Non-diabetic



Thank you ...

