



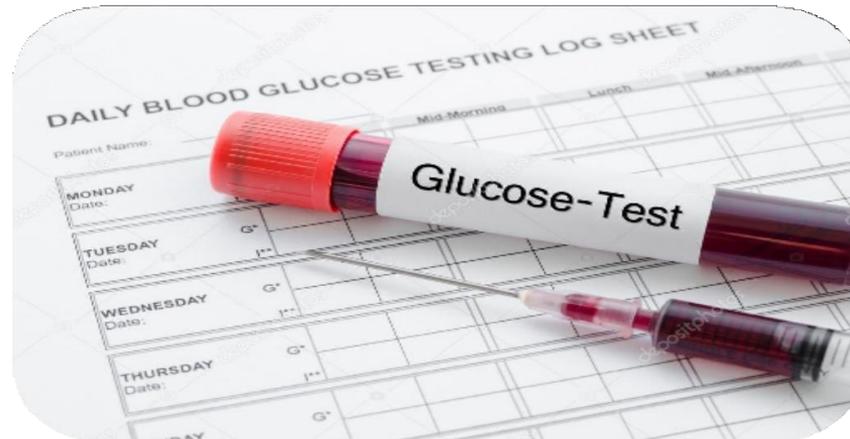
Mansoura National University Faculty of Medicine

Level: 2

Semester: 3



Laboratory diagnosis of DM





Contents



- 1. Biochemical basis of diabetes mellitus**
- 2. Laboratory tests for diagnosis of DM**
- 3. Colorimetric estimation of blood glucose**



Learning Outcomes (LOs)



At the end of this section, the students should be able to:

- 1 Define **diabetes mellitus** and distinguish its two types
- 2 Recognize different laboratory tests needed for **diagnosis** of DM
- 3 Describe **oral glucose tolerance test (OGTT)**
- 4 Identify the principle of laboratory **estimation of blood glucose**, perform it and interpret the result
- 5 **Correlate** the knowledge to a case of Diabetes Mellitus



Case scenario (Clinical correlate)



A 14-year-old male student was seen by his physician. He complains of weight loss and **increase in appetite, thirst, and frequency of urination**. His uncle was diabetic.

- **What is your provisional diagnosis?**
- **Suggest the suitable biochemical tests to perform**





Biochemical basis of diabetes mellitus



Diabetes mellitus (DM) is a metabolic disorder of carbohydrate, lipid and protein metabolism characterized by **hyperglycemia**, which may be due to:

- defect in insulin secretion by pancreatic β cells (**type 1 DM**) or
- insulin resistance in peripheral tissues (**type 2 DM**).



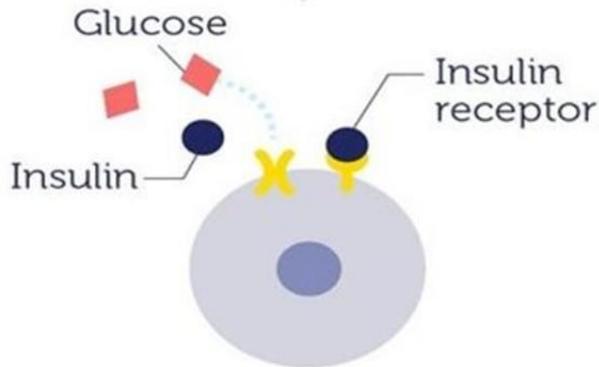
Biochemical basis of diabetes mellitus



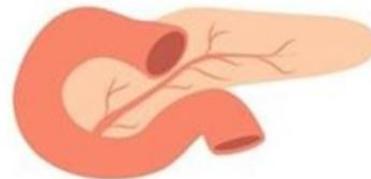
Healthy



Pancreas



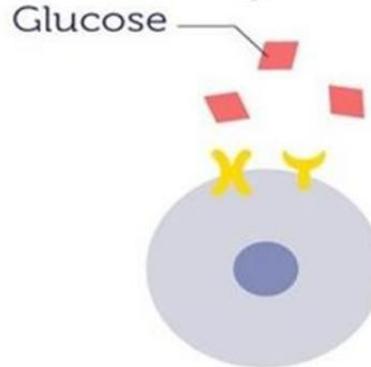
Type 1



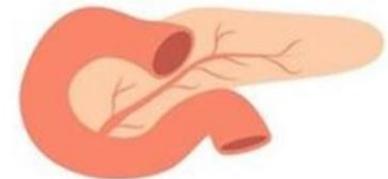
Pancreas



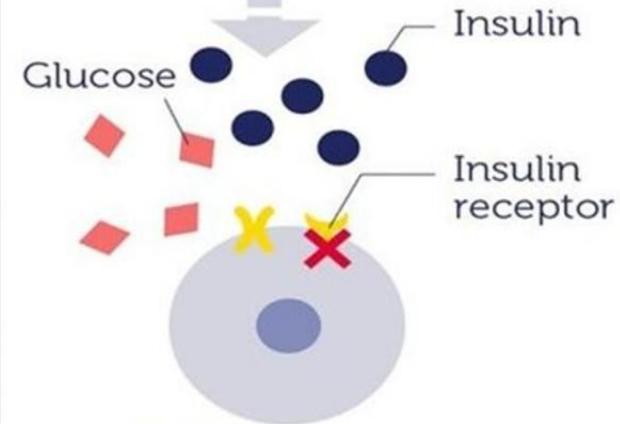
Pancreas fails to produce insulin



Type 2



Pancreas



Cells fail to respond to insulin properly



Laboratory tests for diagnosis of DM



1. Fasting blood glucose
2. Random blood glucose
3. Oral glucose tolerance test
4. Hemoglobin A1c
5. Urine microalbumin test (for diabetic nephropathy).



Blood glucose levels



Normal blood sugar levels chart



Blood sugar classification	Normal	Prediabetes	Diabetes
Fasting	70-100mg/dL	101-125mg/dL	125mg/dL and above
PP (Post Meal Blood sugar level after 2 hrs)	70-140mg/dL	141-200mg/dL	200mg/dL and above
Random blood sugar level	70-140mg/dL	140-200mg/dL	200mg/dL and above

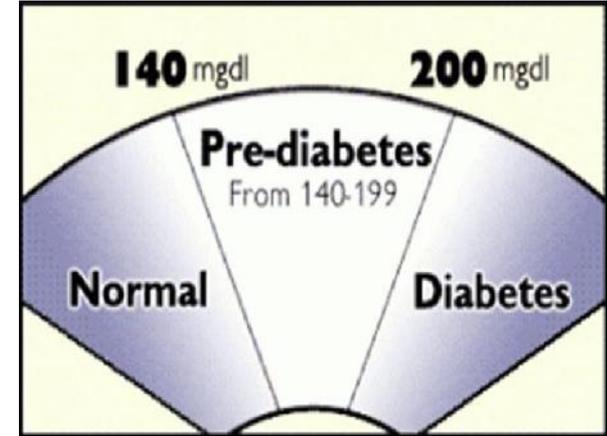


Blood glucose levels



Impaired glucose tolerance:

- It is a **transition phase** between normal glucose tolerance and diabetes.
- It is also referred to as **pre-diabetes** because people with IGT have a higher risk of developing diabetes.
- In impaired glucose tolerance (IGT), blood glucose levels are **between normal and diabetic**.





Oral Glucose Tolerance Test (OGTT)



- Glucose tolerance means the **ability of the body to utilize a given load of glucose.**
- A zero-time **blood** and **urine** samples are taken after **8 hours fasting** for glucose determination.





Oral Glucose Tolerance Test (OGTT)



- The patient is then given a measured dose of glucose solution to drink
- Blood and urine samples are collected at **30 minutes** intervals over a period of **2.5** hours for glucose measurement.
- Results are graphed against time.

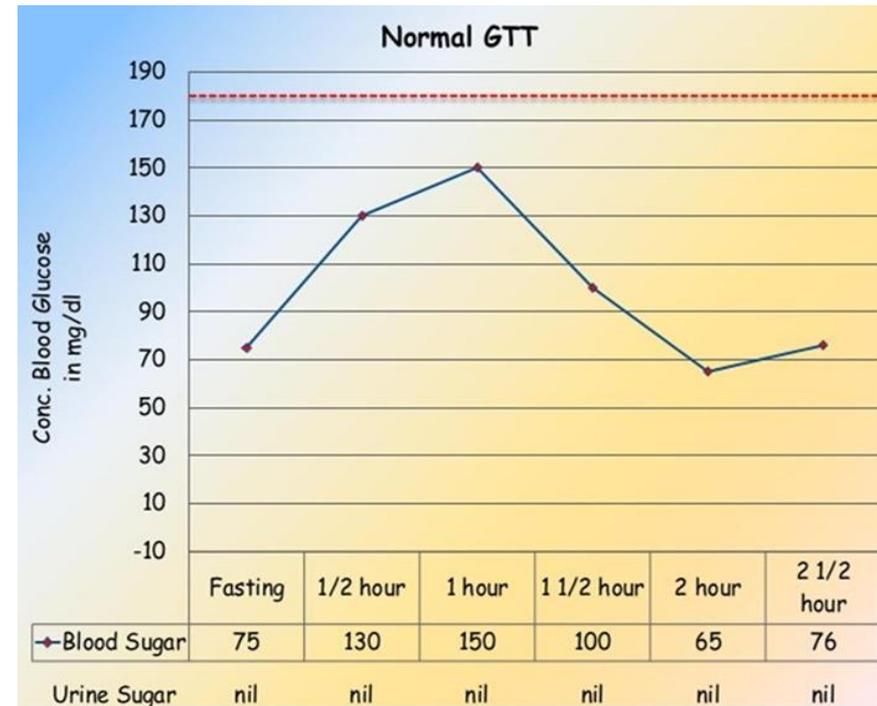




Normal Glucose Tolerance Curve



- **At the start:** Fasting blood glucose is within the **normal** range 70-100 mg/dl.
- **At about one hour:** Blood glucose level **rises to a peak** (since the rate of glucose absorption exceeds its rate of utilization).
- **After 1 hour:** Blood glucose **begins to fall** (as a result of uptake & utilization of glucose by the **tissues**).





Normal Glucose Tolerance Curve



- **After 2 hours:** blood glucose returns to normal level.
- **In the course of return to normal,** blood glucose frequently falls below the initial level due to overshooting of insulin production.
- Blood glucose never exceeds the renal threshold and **no glucosuria** occurs.

N.B. Renal tubules can reabsorb glucose from glomerular filtrate up to the level of 180 mg/dl in blood (**Renal threshold**).

Glucose appears in urine when blood glucose increases beyond **180 mg/dl (renal threshold)**

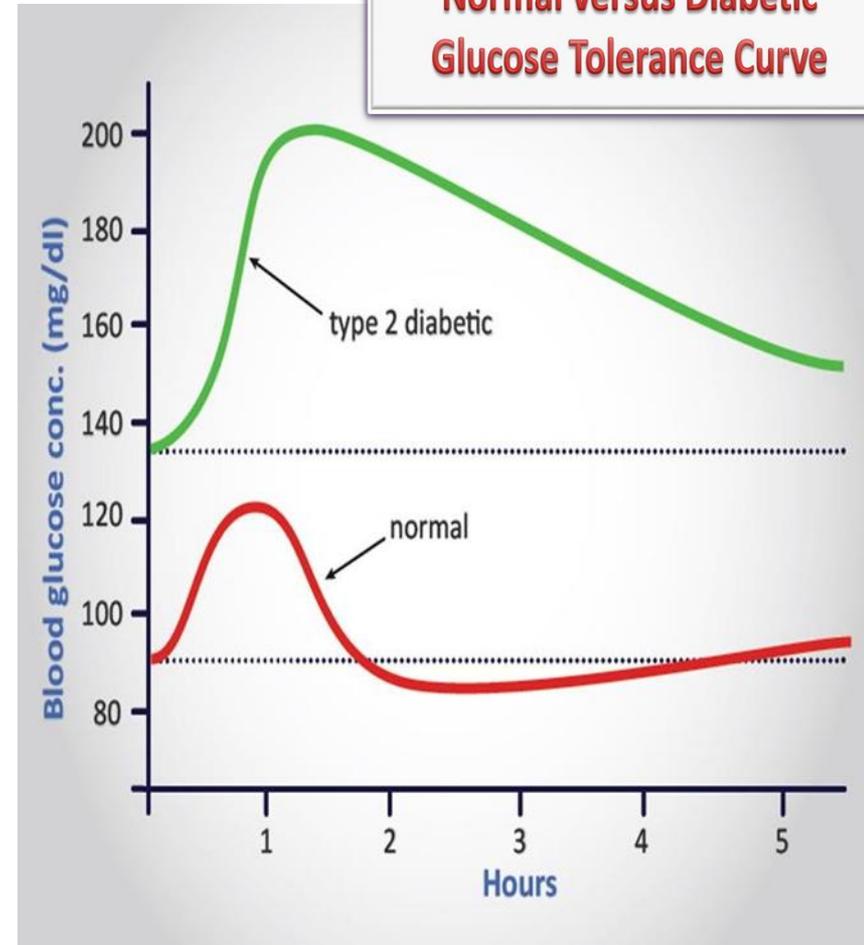


Diabetic glucose tolerance curve



Normal versus Diabetic Glucose Tolerance Curve

- **Fasting blood glucose is higher than the normal limit**
- **After glucose ingestion, blood glucose level will rise higher and may exceed renal threshold (>180 mg/dl) leading to **glucosuria**.**
- **The blood glucose remains high for a longer time (the curve is more flat).**

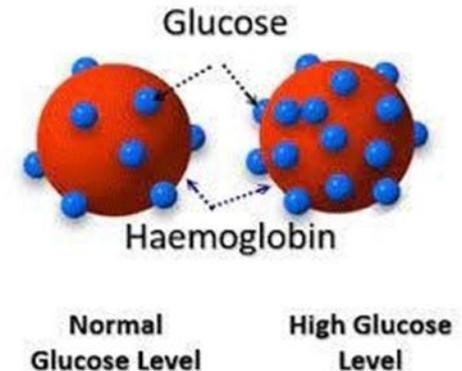




Glycated hemoglobin (HbA1c)



- Hemoglobin A1c is a form of hemoglobin that is **non-enzymatically** linked to glucose (**glycated hemoglobin**).
- HbA1c test measures the amount of blood glucose attached to hemoglobin.

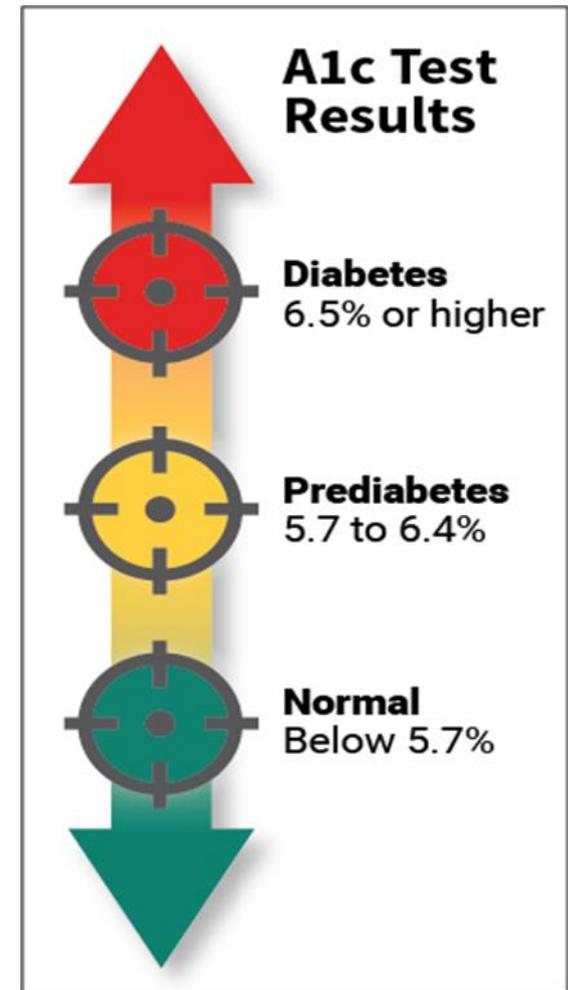




Glycated hemoglobin (HbA1c)



- HbA1c gives a hint about blood glucose levels for the last **2 - 3 months** (RBCs life span is about 90-120 days).
- HbA1c normal range is between **4%** and **5.6%**.



Criteria for diagnosis of DM



Fasting plasma glucose

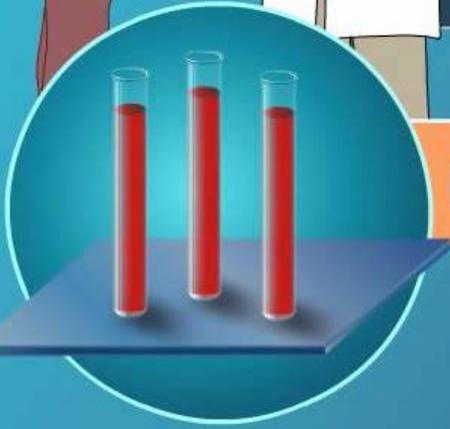
126 mg/dL or higher on 2 separate occasions

HbA1c

6.5% or higher on 2 separate occasions

Oral glucose tolerance test (OGTT)

2-hour post OGTT blood glucose 200 mg/dL or higher



FBG, HbA1c or OGTT high on 2 occasions



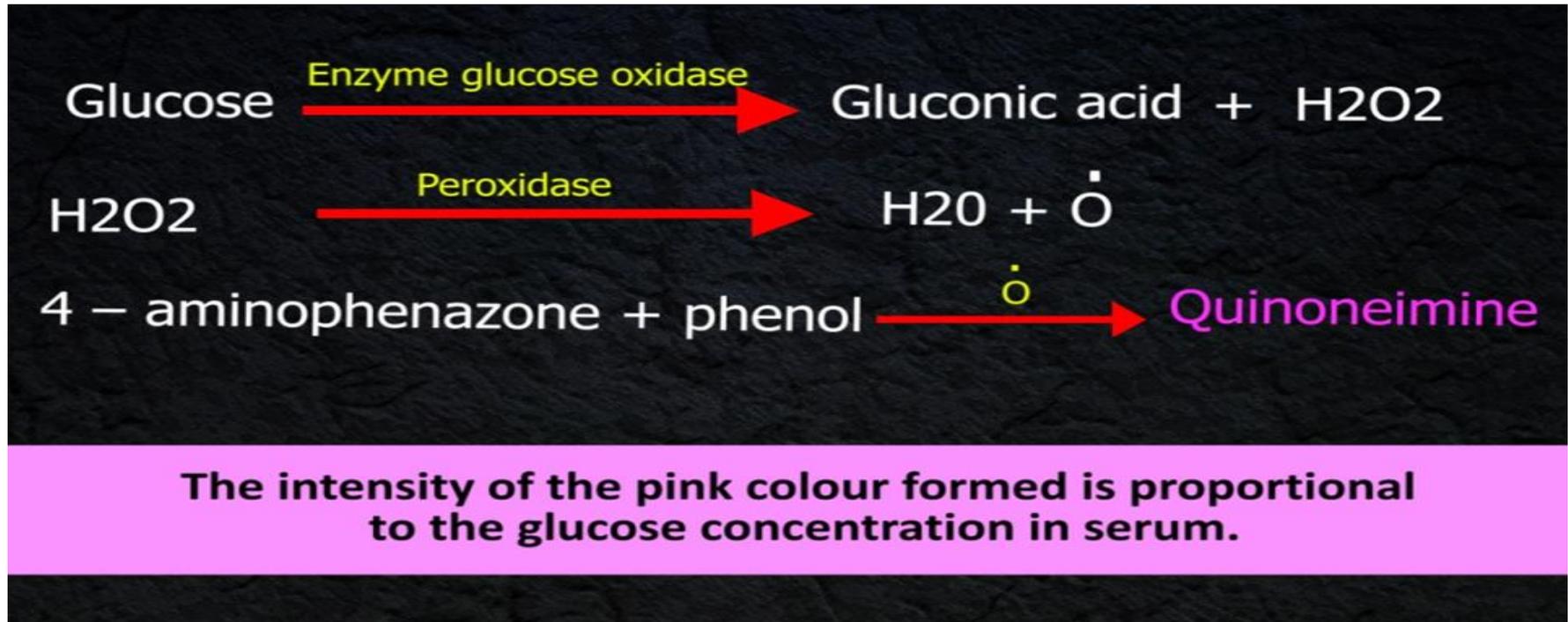
DIABETES



Estimation of blood glucose



Principle: glucose oxidase method



Methods

1. Glucometer:

- Simple, easy, regular checkup for blood glucose



2. Colorimeter:

- Commonly used in the clinical laboratories





Colorimetric estimation of blood glucose



- A colorimeter is used to determine the **concentration** of compounds in solution.
- Certain reagents are added on the biological samples to produce **colored products** when certain compounds are present in the sample.
- By measuring the **absorbance** of the colored products at a specific wavelength of light ,the concentration can be calculated.

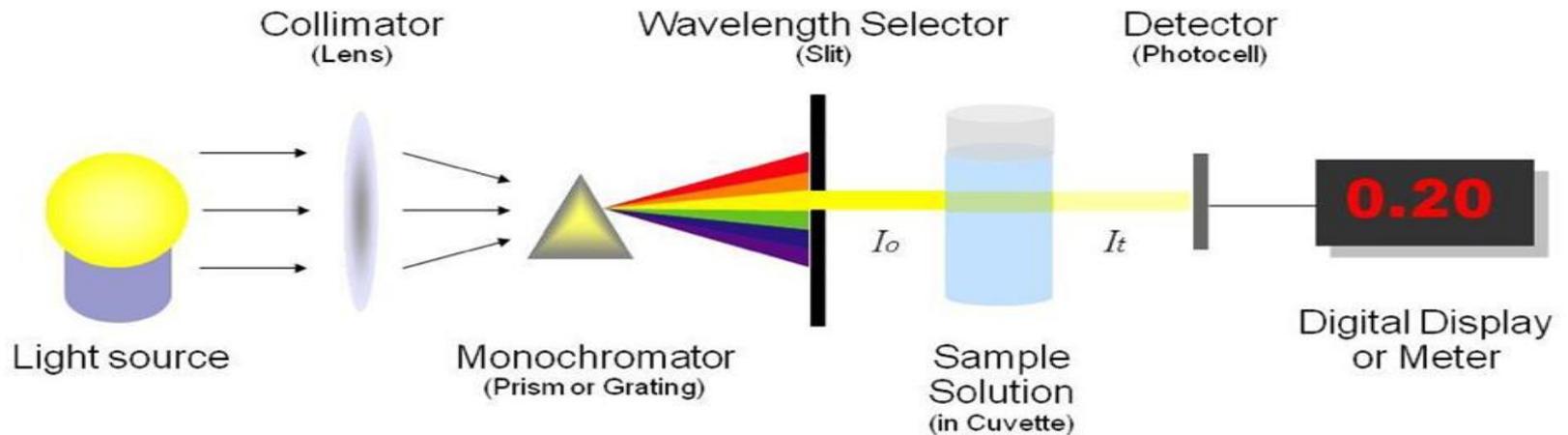




Colorimetric estimation of blood glucose



- N.B: colored solutions can **absorb** lights at certain wavelengths.
- The amount of light absorbed is **proportional** to the solute concentration present in solution.





Colorimetric estimation of blood glucose



Sample: serum, plasma (fasting & 2 hour postprandial samples).

Procedure: pipette into test tubes

	Blank	Standard	Sample
Working reagent	1 ml	1 ml	1 ml
Standard		10 μ l	
Sample			10 μ l
Distilled water	10 μ l		

Mix & incubate for 20 minutes at room temperature or 10 minutes at 37°C



Colorimetric estimation of blood glucose



Reading:

Measure the absorbance of sample and standard against blank at wavelength 505 nm (490-550 nm).





Colorimetric estimation of blood glucose



Calculation

$$\text{Concentration of Glucose} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard} = \dots \text{ mg/dl} \quad (100\text{mg/dl})$$



Hyperglycemia

Definition:

increased **fasting** blood glucose level more than normal level (125 mg/dl and above) or increased **2h postprandial** glucose (200 mg/dl and above).

Causes:

1- **Deficiency of insulin:** as in D.M.

2- **Increase of anti-insulin hormones:**

- **Adrenaline**
- **Cortisol**
- **Thyroid hormone**
- **Growth hormone**





Case report



- A possible diagnosis is **type I Diabetes**.
- **Initial biochemical tests to be done include:**
 1. **Fasting Blood Glucose**
 2. **2h postprandial OGTT blood glucose**
 3. **HbA1c**



Thank You