Blood Glucose Test

Enzymatic Colorimetric Method

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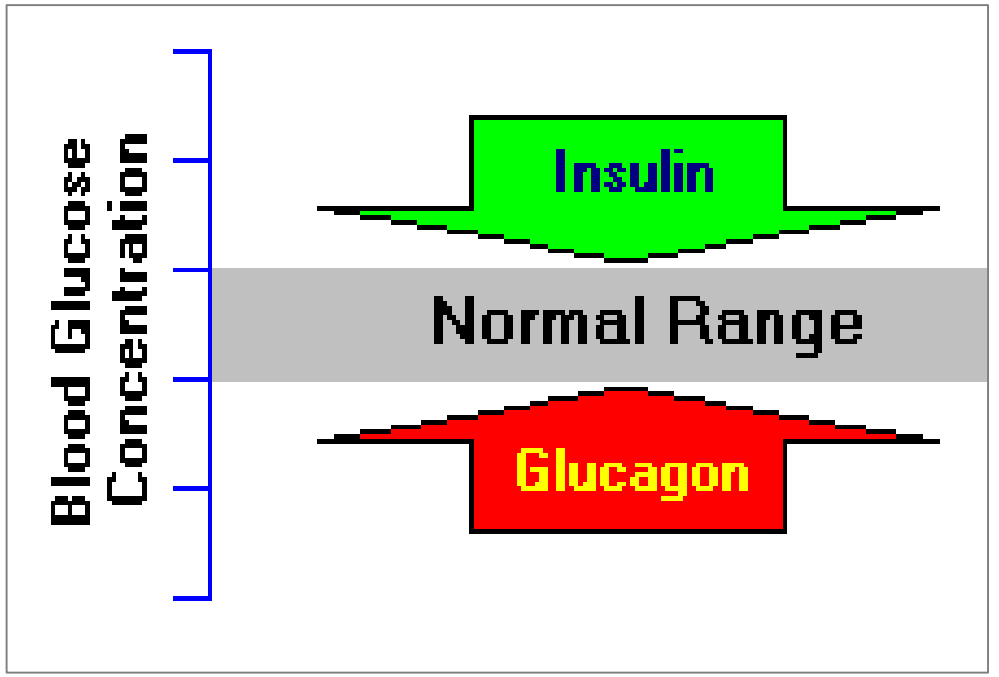
Intended learning outcomes

- Identify the principles of the blood glucose test
- Calculation of glucose concentration in the unknown sample

Introduction

- Glucose is monosaccharide found in fruits and also derived from the breakdown of carbohydrates in the diet & the conversion of glycogen by liver.
- Glucose is the main source of energy used by the body cells.
- The two hormones that directly regulate blood glucose are Glucagon and Insulin.



Regulation of blood glucose

Glucose Homeostasis

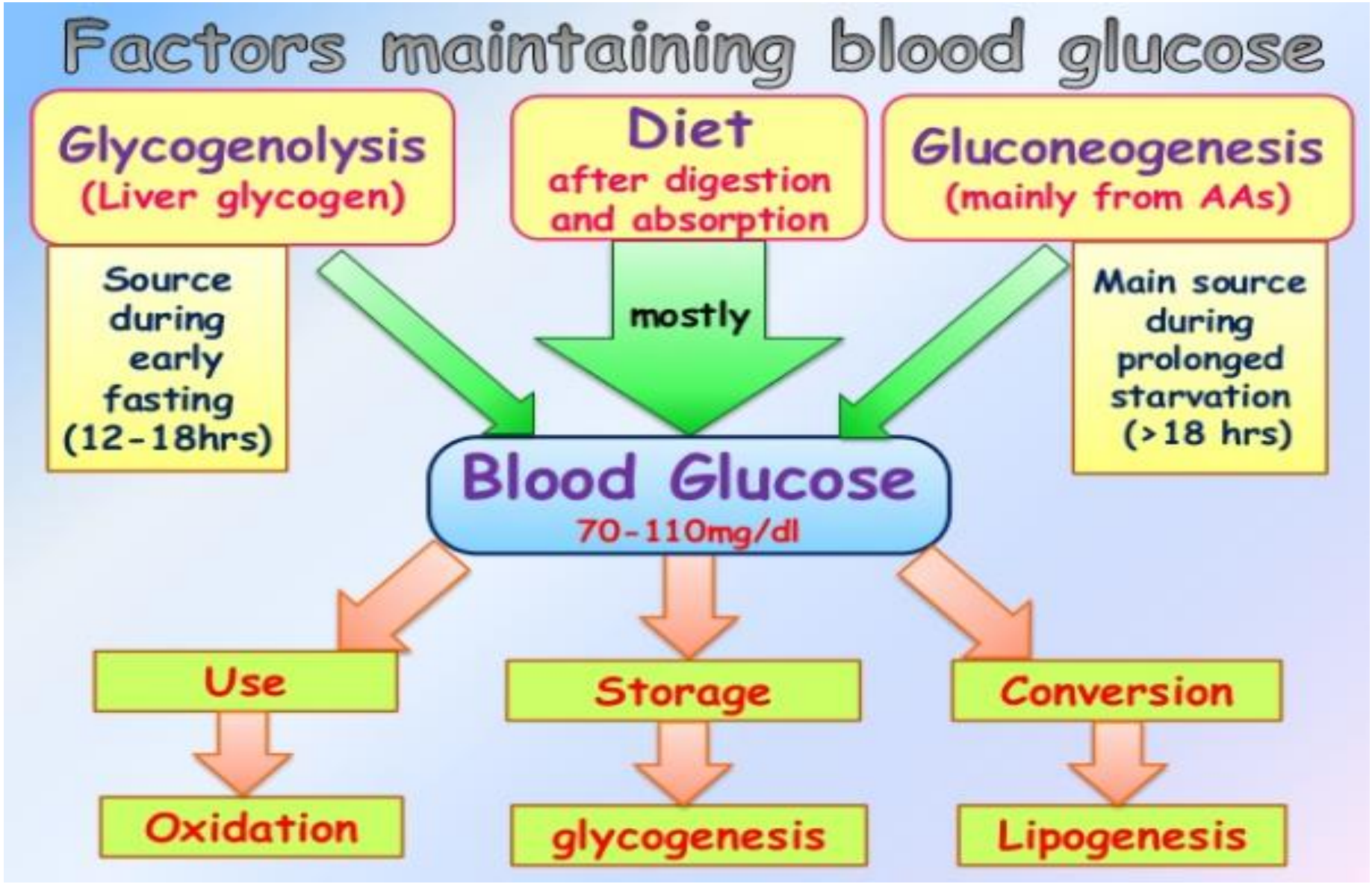
- It is the maintenance of blood glucose level within the normal range.
- The blood glucose level must be maintained within the narrow limits of 70-100 mg/dl.

Regulation of blood glucose

- Normal blood glucose level (fasting) is **70-110 mg/dl**
- Post-prandial blood glucose level is **120-140 mg/dl**
- Above and below these levels is considered as abnormal.

➢ **Hyperglycemia** - Levels above the normal range

➢ **Hypoglycemia** - Levels below the normal range


Factors maintaining blood glucose
Glycogenolysis
(Liver glycogen)
Diet
after digestion
and absorption
Gluconeogenesis
(mainly from AAs)
Source during early fasting (12-18hrs)
mostly
Main source during prolonged starvation (>18 hrs)
Blood Glucose
70-110mg/dl
Use
Storage
Conversion
Oxidation
glycogenesis
Lipogenesis

Homeostasis of blood glucose

- **Low blood glucose** ⟹ **Alpha cells release glucagon** ⟹

  Glycogenolysis in the liver; Glucose released to blood ⟹

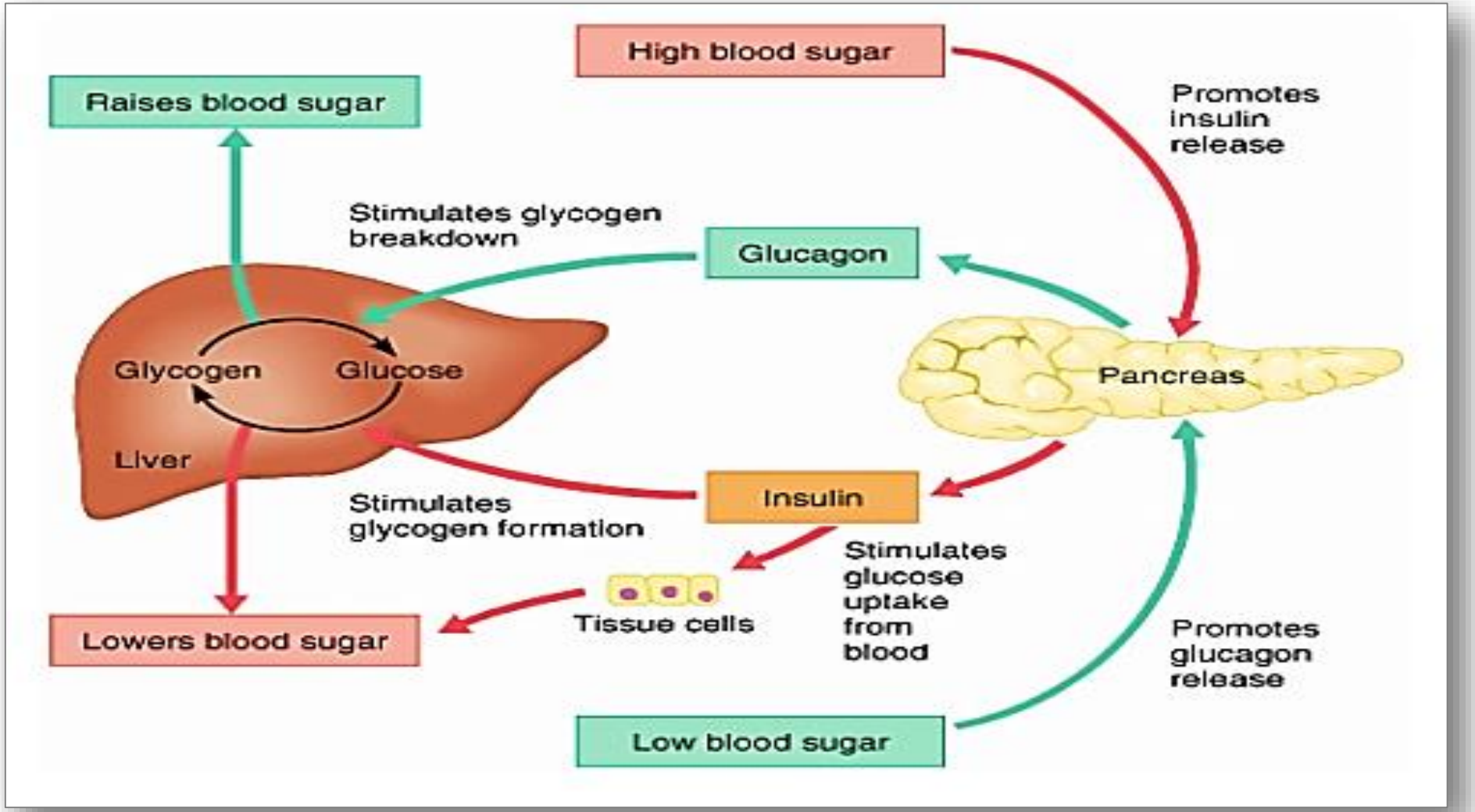
  Normal blood glucose

- **High blood glucose** ⟹ **Beta cells release insulin** ⟹

  Peripheral tissue Cells take glucose From blood ⟹

  Normal blood glucose

Blood sugar insulin cycle graphic



Insulin

- The insulin plays an important role in storing the excess energy.
- In the case of excess carbohydrates, it causes them to be stored as glycogen mainly in the liver and muscles.
- All the excess carbohydrates that cannot be stored as glycogen are converted under the stimulus of insulin into fats and stored in the adipose tissue.

Insulin

- Driving of insulin into the cell requires **insulin receptors**.
- After meal the pancreas releases insulin for **glucose metabolism** provided there are enough insulin receptors
- Insulin bind to these receptors on the surface of target cells such as found in fat and muscle, this opens the channels so that glucose can pass into cells, where it can be converted to energy.
- As cellular glucose metabolism occurs, blood glucose level falls

Methods of analysis

- In the past, the majority of the quantitative test for glucose determination depended upon the oxidation of glucose by hot, alkaline copper solutions or solutions of potassium ferricyanide.
- These were replaced by the ortho-toluidine test and later by enzyme methods.
- **Enzymatic methods** give “true” glucose determination because of the high specificity of an enzyme for a particular substrate and don’t consume the time.

Principle

- Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid .
- The formed hydrogen peroxide (H_2O_2) reacts under catalysis of peroxidase with phenol and aminophenazone to a red - violet Quinone dye as indicator.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{aminophenazone} \xrightarrow{\text{POD}} \text{Quinone} + H_2O$$

Red – violet

Specimen collection

- **Serum, plasma is suitable for samples.**
- **Whole blood and hemolysis are not recommended for use as a sample.**
- **Freshly drawn serum is the preferred specimen.**
- **Stability: serum , heparin or EDTA plasma (with addition of glycolytic inhibitor):**

❖ **2 days at 20-25°C**

❖ **7 days at 2-8 °C**

Procedure

Reagent Preparation

- Working reagent (WR) dissolve the content of one vial R2 enzyme in one bottle of R1 buffer.
- Cap and mix gently to dissolve the contents.

Reagent stability

The reagent is stable 1 month after reconstitution in the refrigerator (2-8)°C or 7 days at room temperature (15-25) °C .

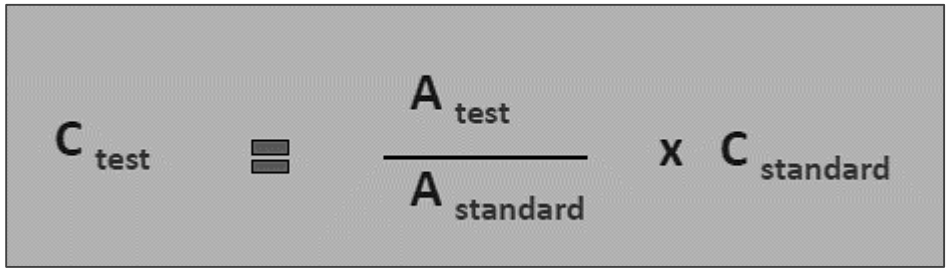
Procedure

Pipette in to cuvettes	Blank	STD	Sample
WR	1ml	1ml	1ml
STD	-	10µl	-
Sample	-	-	10µl

- Mix and incubate for 10 min , at 37 °C or 20 min, at 20-25 °C
- Read the absorbance (A) of the samples and standard, against the Blank (ΔA) at 505 (490-550) nm.
- Colour is stable for 60 minutes

Concentration of glucose

Use **Beer-Lambert equation** to receive to the concentration of glucose in the patient sample:



Conversion factor: mg/dL x 0.555 = mmol/L.

Conversion factor : mmol/L x 18 = mg/dL.

Normal value: Serum, Plasma (fasting) 60 - 110 mg/dL or 3.33 - 6.10 mmol/L

THANK YOU !

ANY QUESTIONS ??

PLEASE ASK