BCM 101
BIOCHEMISTRY
Week 3 Practical
"Colorimetric determination of blood sugar level"

In medicine, **blood sugar** is a term used to refer to the level of glucose in blood. Glucose, transported via the bloodstream, is the primary source of energy for the body cells. Blood sugar level (BSL), or serum glucose concentration, is tightly regulated in the human body so that its level remains within a certain limit (70 to 150 mg/dl) throughout the day.

The aim of this practical session is to:

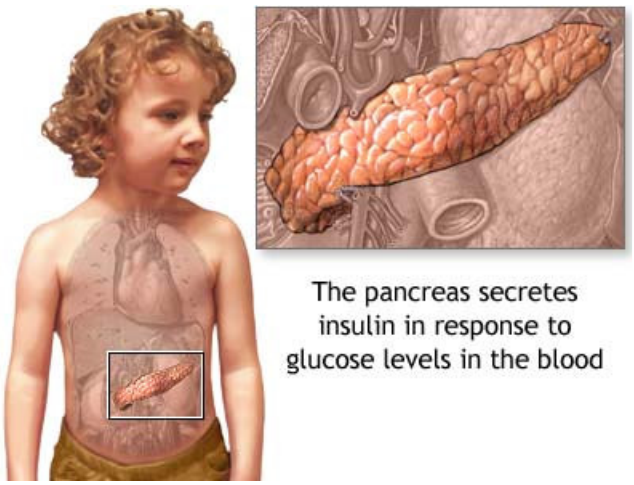
1. Obtain a simplified knowledge about the regulation of BSL and its clinical correlation to diabetes mellitus.
2. Recognize different methods used for the determination of BSL.
3. Determine the BSL in a serum sample of a fasting individual and comment on the case.

Regulation of blood sugar level

BSL is controlled by the following hormones:

1. **Insulin**: it is a polypeptide hormone secreted from the beta cells of the islets of Langerhans in the pancreas; it lowers BSL causing hypoglycemia.

2. **Glucagon** (secreted from the alpha cells of the islets of Langerhans in the pancreas), **epinephrine (adrenaline)**, **corticosteroids** and **GH** raise BSL causing hyperglycemia.



The pancreas secretes insulin in response to glucose levels in the blood

Diabetes mellitus

The term "**diabetes**" is derived from a Greek word that means "excessive urine production", while the term "**mellitus**" is a Latin word that means a "sweet taste".

Diabetes mellitus (DM) is the failure of the body to metabolize carbohydrates properly, together with altered lipid and protein metabolism. It is characterized by **hyperglycemia** (high BSL above normal), **glucosuria** (presence of glucose in urine), **polyuria** (frequent urination), **polydipsia** (increased thirst) and **polyphagia** (increased appetite).

Classification of diabetes mellitus:

The **World Health Organization** (**WHO**) classifies DM into:

Type 1 DM (insulin-dependent diabetes mellitus; IDDM), which is caused by insufficient or non-existent production of insulin.

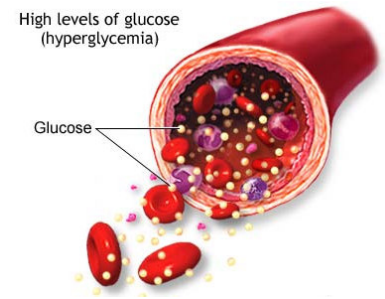
Type 2 DM (non-insulin-dependent diabetes mellitus; NIDDM), which is caused by decreased tissue response to insulin (insulin resistance).

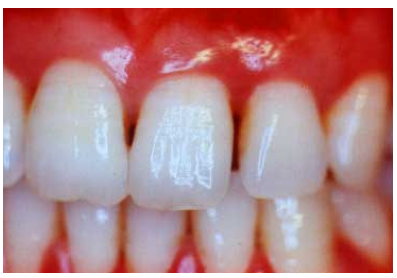
Gestational diabetes, which develops during pregnancy.

Complications of diabetes mellitus:

Diabetes mellitus can cause many **complications** that arise from the prolonged exposure of tissues to elevated glucose concentration. These include:

1. Renal failure.
2. Retinal damage.
3. Nerve damage.
4. Gangrene and amputation.
5. Concerning the field of **dentistry**, studies showed that patients with insufficient blood sugar control seem to develop gum disease more frequently and more severely than people who have good management of their diabetes, which is one of the leading causes of tooth loss among adults.



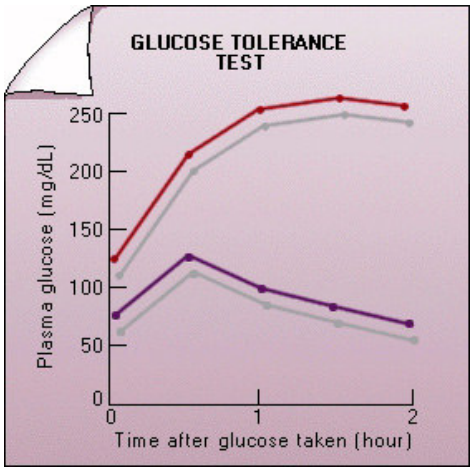


Gum disease

Diagnosis of diabetes mellitus:

DM is diagnosed by demonstrating either of the following:

1. **Fasting BSL** at or above 126 mg/dl:
   N.B. The normal fasting BSL is 70-110 mg/dl; therefore, values between 110 -126 mg/dl indicate impaired fasting BSL and prediabetes.
2. BSL at or above 200 mg/dl two hours after a standard oral glucose load in an **oral glucose tolerance test** (**OGTT**).



Methods for determination of blood sugar level

1. Enzymatic methods:

e.g. **Glucose oxidase method**:

$$\text{Glucose} \xrightarrow{\textbf{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$2\,H_2O_2 \xrightarrow{\textbf{Peroxidase}} 2\,H_2O + O_2$$

$$\text{o-toluidine} + O_2 \longrightarrow \text{Colored product}$$

The colored product is then measured colorimetrically (as described later).

2. Chemical methods:

a. Oxidation-reduction reaction (alkaline copper reduction method):

This method depends on the reducing properties of glucose which reacts with cupric ions in alkaline medium producing the red colored cuprous oxide that can be measured colorimetrically (as described later).

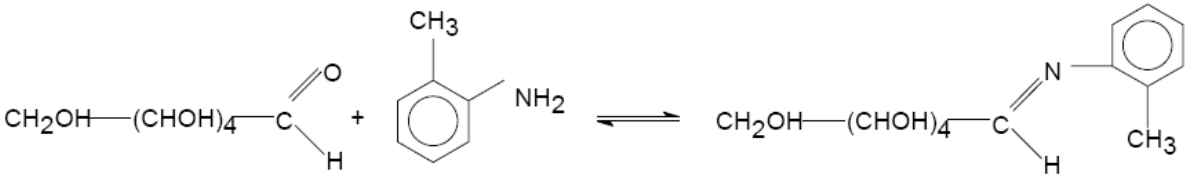
$$\text{Glucose} + Cu^{2+} \xrightarrow{\text{Alkaline medium}} Cu_2O \text{ (red color)}$$

b. Condensation reaction (o-toluidine method).

Colorimetric determination of BSL using the o-toluidine method

Principle:

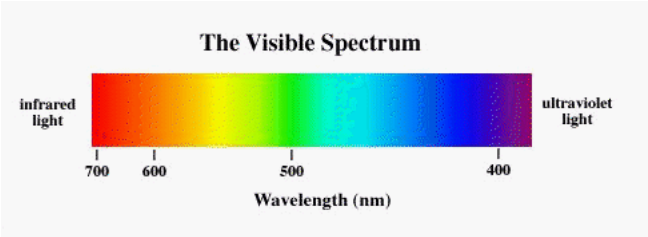
In this method, o-toluidine reacts in hot glacial acetic acid with the terminal aldehyde group of glucose to produce a blue-green colored condensation product that can be measured colorimetrically at λ_{max} 630 nm.



glucose o-toluidine blue-green complex

The colorimeter:

The **colorimeter** is a device used to measure the absorbance of a colored solution at a particular wavelength of light (400-700 nm) which is the visible region.



Colorimeters rely on the principle that the absorbance of a substance is directly proportional to its concentration, i.e. a more concentrated solution gives a higher absorbance reading.

To estimate BSL, one of the two following methods can be used:

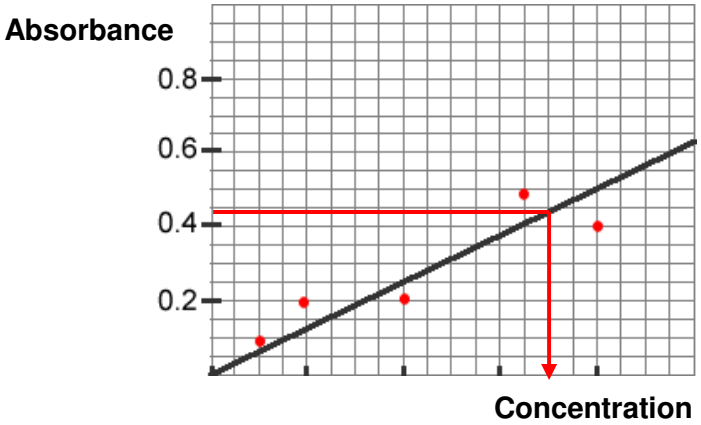
1. Performing the **o-toluidine** method on a "**standard"** glucose solution (i.e. of known concentration) and then applying the following equation:

$$\frac{C_{test}}{C_{std}} = \frac{A_{test}}{A_{std}}$$

$$\therefore C_{test} = C_{std} \times A_{test} / A_{std}$$

Where, C_{test} is the glucose concentration in the test solution.
C_{std} is the glucose concentration in the standard solution.
A_{test} is the absorbance of test solution.
A_{std} is the absorbance of the standard solution.

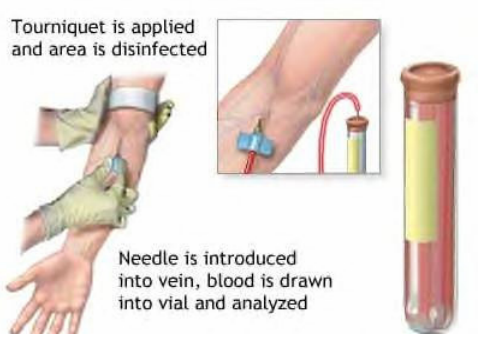
2. Performing the **o-toluidine** method on a "**series**" of standard glucose solutions and then constructing a "**standard curve**" by plotting the absorbance on the y-axis and the concentration on the x-axis. From this curve, the absorbance reading of any sample can be converted into concentration.



Practical:

1. Blood is drawn from a vein and transferred into a centrifuge tube.
2. Serum is obtained by centrifugation of blood for 10 minutes. The **centrifuge** is a device that puts an object in rotation around a fixed axis and applies force perpendicular to this axis.

   The centrifuge works using the sedimentation principle, where it is used to separate lighter and heavier substances.



Centrifuge

3. Determine the glucose concentration in the provided serum sample of patient **1, 2** or **3** using the **o-toluidine** method as follows:

   - In a clean dry test tube, add 0.1 ml of distilled water (blank) or standard glucose (standard) or serum (test), then add 2 ml of o-toluidine reagent.

	Blank	**Standard**	**Test**
Distilled water	0.1 ml	—	—
Standard	—	0.1 ml	—
Test	—	—	0.1 ml
o-toluidine	2 ml	2 ml	2 ml

- Mix the content of each tube.
- Cover the tube opening with aluminium foil.
- Put the tubes in a boiling water bath for 10 minutes.
- Remove test tubes from the water bath and cool under tap water.
- Read the absorbance at λ_{max} 630 nm.
- Calculate the concentration of BSL in the provided **fasting blood samples** using the absorbance reading of standard glucose and applying the following equation:

$$C_{test} = C_{std} \times A_{test} / A_{std}$$

- **Comment on the case provided**:

  1. Normal (fasting BSL = 70 – 110 mg/dl)

  2. Prediabetic patient (fasting BSL between 110 and 126 mg/dl)

  3. Diabetic patient (fasting BSL at or above 126 mg/dl).

  4. Hypoglycemic patient (below 70 mg/dl).

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Student Name: ..... **Student ID:** .....

Laboratory exercise:

1. Determine the blood sugar level (BSL) in the provided fasting serum sample using the O-toluidine method.

Calculations & Results:

Patient number ……..

A_{test} = …….

C_{std} = …….

A_{std} = …….

$C_{test} = C_{std} \times A_{test} / A_{std}$

= ……

2. Write your **comment** on the case:

……

……

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