

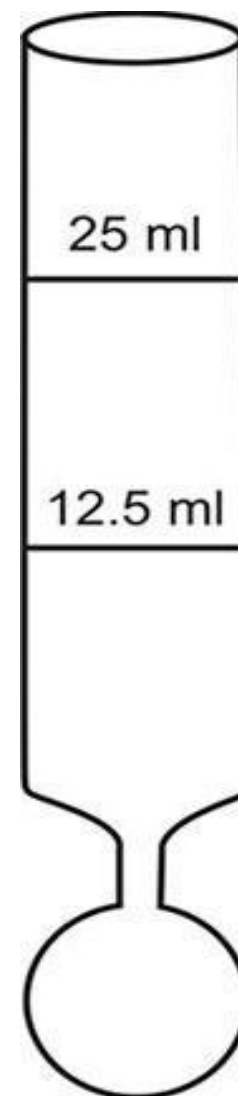
Folin & Wu method

- **Reagents**

- 10% sodium tungstate
 - 2/3 N H_2SO_4
 - Alkaline Copper Sulphate solution
 - Phosphomolybdic acid
 - Standard glucose solution(.2mg/2ml)
- for preparing
Plasma free filtrate

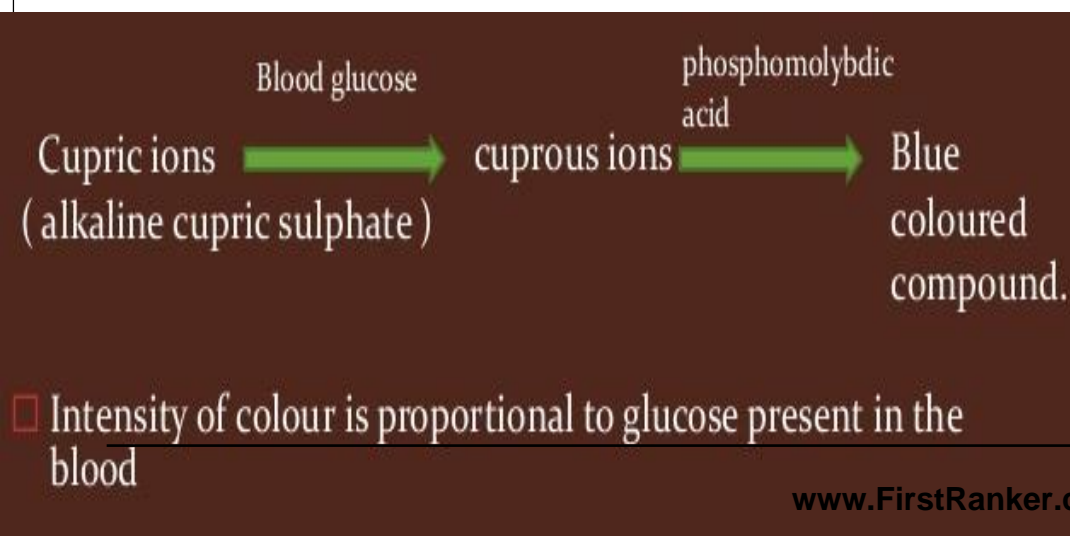
Folin-Wu test tube

- This is special type of test tube .
- The constricted part of the tube prevents the entry of O_2 to the bulbar portion of test tube and thus prevents the re oxidation of cuprous oxide present in the bulb of tube.



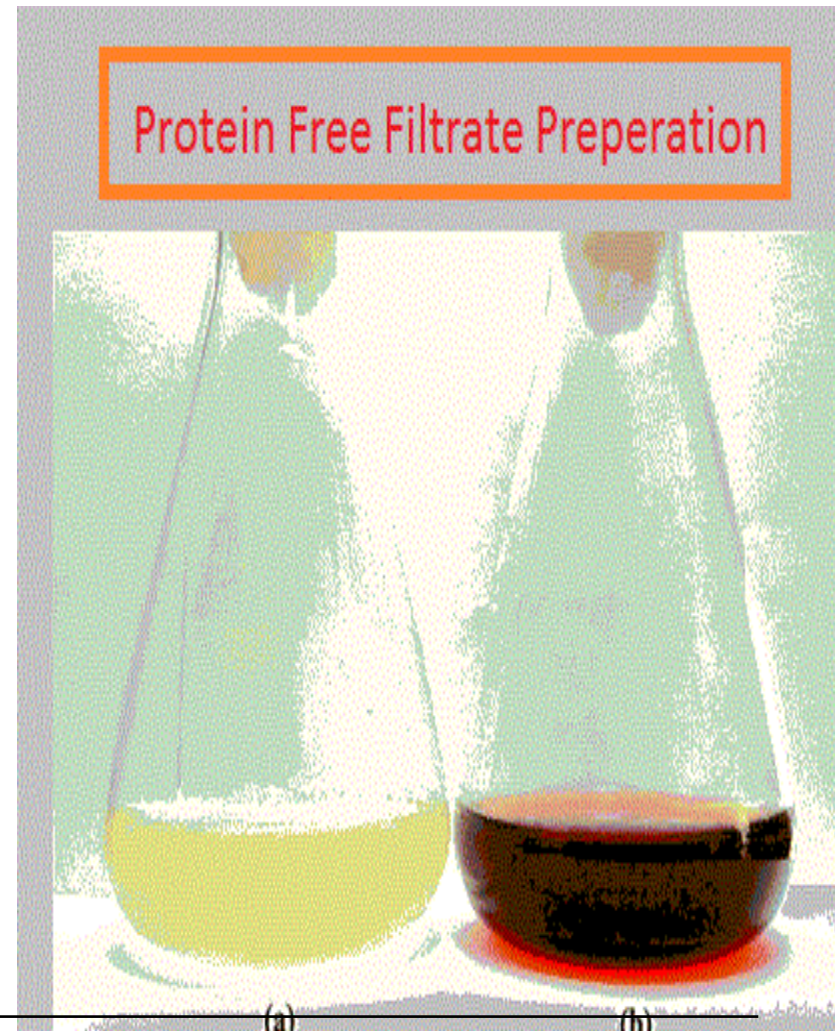
Principle

- Glucose and other reducing substances reduce cupric to cuprous ions, which on reaction with the phosphomolybdic acid produces a blue color. The blue color is produced due to reduction of molybdate ions.
- The intensity of color is directly proportional to amount of reducing substance which is to be determined.



Protein free filtrate(PFF)

- Protein free filtrate is prepared by taking 2ml of oxalated blood.
- Add 14ml distal water
- 2ml of 10% sodium tungstate.
- 2ml of $2/3$ N H_2SO_4
- Shake well,
- keep at room temperature for 10-15min until color change to brown red then filter it clear fluid is PFF.
- 2ml of blood is diluted 10 time, so in calculation we add dilution factor 10.



Procedure

- Label 3 folin wu test tubes as
- Unknown(U), standard (S), and blank(B).

No.	REAGENTS.	TUBES.		
		U	S	B
1.	P.F.F.	2ml	-	-
2.	Standard Glucose Solution.	-	2ml	-
3.	Distilled Water.	-	-	2ml
4.	Alkaline Copper Sulphate Solution.	2ml	2ml	2ml
5.	Keep in BOILING water bath. Remove after 8 minutes and cool under running tap water.			
6.	Phosphomolybdic Acid.	2ml	2ml	2ml
7.	After 2 minutes, dilute with distilled water up to 25ml mark.			
8.	Record the Optical Density at 420nm.			

Calculation

Optical Density of Unknown (U)	=	OD _U
Optical Density of Standard (S)	=	OD _S
Concentration of Standard Solution	=	C _S
PFF Dilution Factor	=	D
Volume of PFF Used.	=	V

$$\text{BLOOD GLUCOSE (mg/dl)} = \frac{\text{OD}_U \times C_S}{\text{OD}_S} \times \frac{D}{V} \times 100$$

NORMAL RANGE:

Fasting Blood Glucose = 65-110 mg/dl
Random Blood Glucose = Up to 140 mg/dl

$$\begin{aligned} \text{Blood glucose} &= 25 \times .2 / 22 \times 10 / 2 \times 100 \\ &= 113.6 \text{ mg/dl} \end{aligned}$$

GLUCOSE TOLERANCE TEST

Dr Gulnaz Begum

GLUCOSE TOLERANCE TEST

- This test is used to determine the ability of body to tolerate or utilize glucose in circulation.

It can measure

- Active insulin
- Ability of liver to absorb or store excessive amount of glucose.
- Effectiveness of GIT to absorb glucose.

Significance of GTT

- i) In asymptomatic persons with sustained or transient glycosuria
- ii) In persons with symptoms of diabetes but no glycosuria or hyperglycemia
- iii) Persons with family history but no symptoms or positive blood findings
- iv) In persons with or without symptoms of diabetes mellitus showing one abnormal blood finding
- v) In patients with neuropathies or retinopathies of unknown origin
- vi) In women with H/o having delivered large babies.

Contraindications of GTT

- a) In proven cases of diabetes mellitus the test is not required.
- b) GTT is required only in doubtful cases, it is not recommended for follow up of patient.
- c) The test should not be carried out in acutely ill patients

Types of GTT

- Standard Oral glucose tolerance test
- I/V Glucose tolerance test

in

Patients having the error in absorption from GIT, due to malabsorption syndrome, steatorrhea and caeliac disease.

PREPARATION OF THE PATIENT:

1. Patient should be kept on normal mixed carbohydrate diet for 2 – 4 days before the test.
2. All the drugs, if any being used by the patient, should be stopped.
3. The Patient must have his/her evening meal by 8.00 pm at the latest and should take nothing except plain water, till the test is performed.
4. NO BREAKFAST is allowed in the morning.
5. NO SMOKING should be allowed.

REAGENTS:

1. SAME REAGENTS as for blood Glucose estimation,
2. Benedict's Reagent (For detection of Sugar in urine).

PROCEDURE OF OGTT

- At about 8 a.m. the fasting blood and urine samples are collected. These are called zero samples.
- Administer the prescribed oral glucose solution, usually 75 g of glucose for non-pregnant patients or 100 g for pregnant patients.
- The glucose dissolve in 300 ml of lemon juice/water mixture within 5min after taking sample .



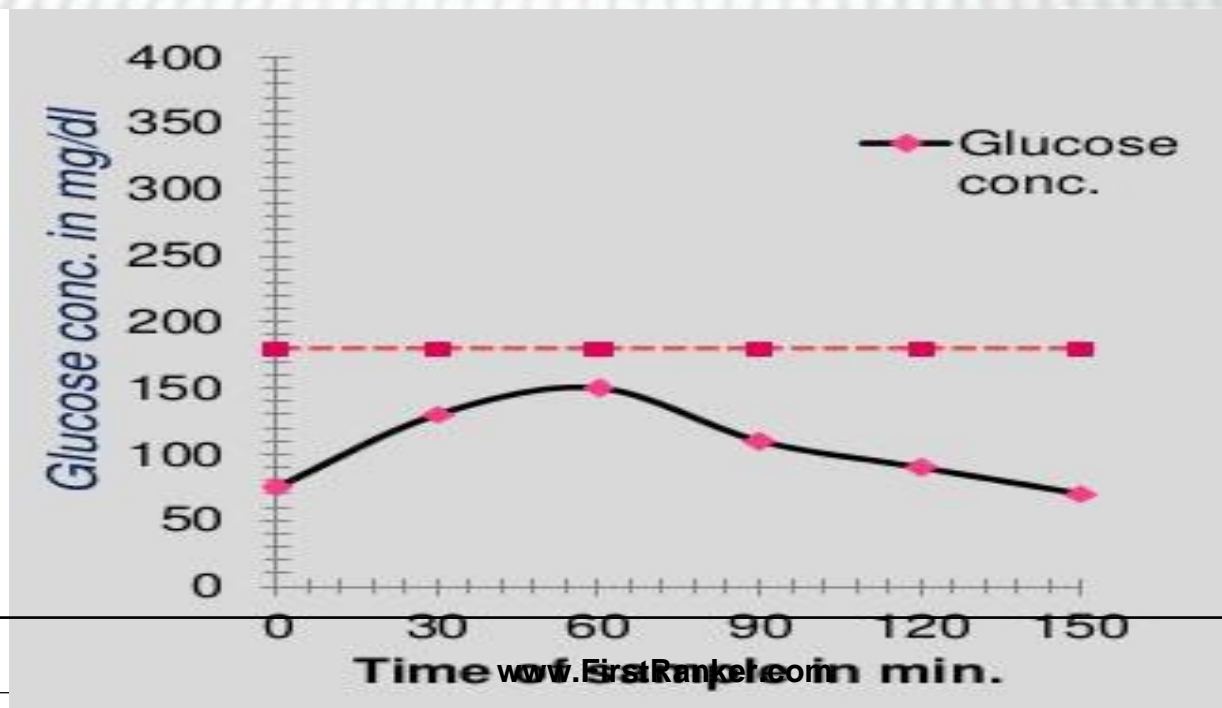
- Instruct the patient to ingest the entire glucose load.
- Tell the patient that he or she cannot eat anything until the test is completed. However, encourage the patient to drink water. No other liquids should be taken during the testing period.
- Inform the patient that tobacco, and smoking are not allowed.
- Collect a venous blood & urine sample at 30 min. interval for upto 150 min.
- Glucose is estimated in all the blood samples.
- ~~Urine is analyzed for the presence of glucose.~~

INTERPRETATION

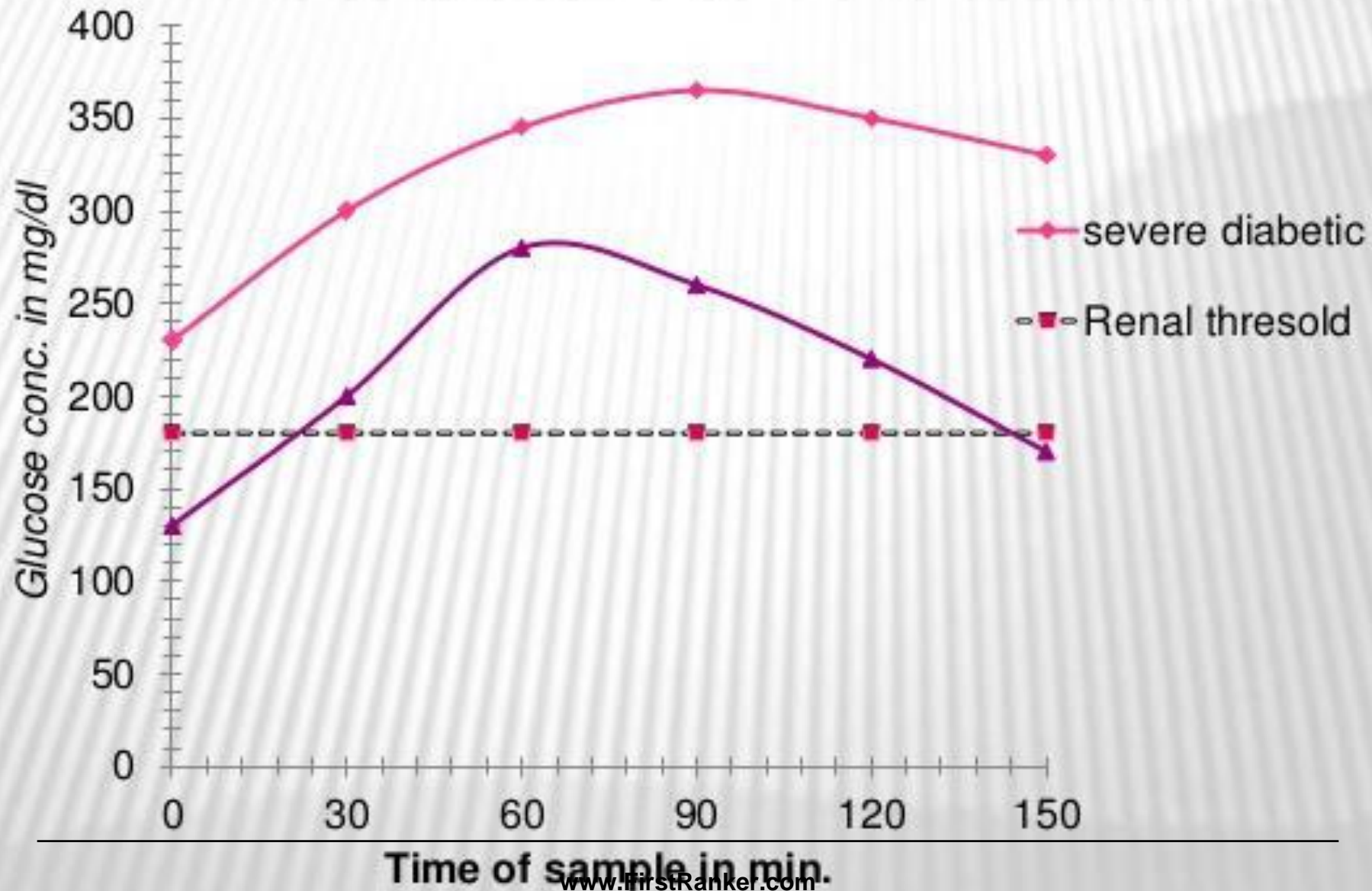
- In normal individuals, blood glucose level returns
- to normal within 60 minutes.
- In diabetes mellitus, decline is slow.
- The initial values are attained in 120 minutes.

Normal GTT

	Fasting	30 min.	60 min.	90 min.	120 min.	150 min.
Blood Glucose (mg/dl)	75	130	150	110	90	70
Urinary Glucose	nil	nil	nil	nil	nil	nil



DIABETIC CURVE



Moderate Diabetic Curve

	Fasting	30 min.	60 min.	90 min.	120 min.	150 min.
Blood Glucose (mg/dl)	130	200	280	260	220	170
Urinary Glucose	nil	++	++	++	++	nil

Severe Diabetic Curve

	Fasting	30 min.	60 min.	90 min.	120 min.	150 min.
Blood Glucose (mg/dl)	230	300	345	365	350	330
Urinary Glucose	++	+++	+++	+++	+++	+++

CLINICAL SIGNIFICANCE

Decrease Glucose Tolerance

- **Diabetes mellitus (DM):** *This disease is defined by glucose intolerance and hyperglycemia.*
- **Acute stress response**
- **Cushing syndrome**
- **Chronic renal failure**
- **Glucagonoma**
- **Acute pancreatitis**
- **Diuretic therapy**
- **Corticosteroid**
- **Myxedema**
- **After gastrectomy.**

GLUCOSE TOLERANCE

Increased Glucose Tolerance

- Increased carbohydrate tolerance is observed in all conditions that cause hypoglycemia:-
- Hypopituitarism
- Hyperinsulinism
- Hypothyroidism
- Adrenal Cortical Hypofunction