(2) Fehling's Test :

⇒ . Fehling soln. in T.T. + Boil
No change in colour
Add glucose + Boil

Result : Yellow / Brick red ppt
Brozof [Cu_2O]

(3) Benedicts' test :

⇒ Benedicts in T.T. + glucose
+ heat + Boil
then cool under tap water

Result Green, Yellow of Red ppt
due to [Cu_2O]

Biochemical Tests

① Test on Carbohydrates
(monosaccharides)

↓

Experiments with glucose

① Reduction of methylene blue

=> D.W. in Test tube + a drop of methylene blue (1%.) → Blue colour
+ 40%. NaOH + Boil =>
(colour not discharged)
+ .2%. glucose + Boil

Result → Solution decolourised

Because [Formation of leuco — methylene blue)

(4) Picric acid test:

~~Test~~ glucose in T-T.

+

add ~~add~~ picric acid

+

40% NaOH

Result → Red colour

Because of picramic acid

presence of carbohydrates

Experiment (1) : **Reduction of methylene blue.**

Procedure : In a test tube take 3 cc of distilled water, then add a drop of methylene blue (1%). The water becomes blue coloured. Add 0.5 cc of 40% NaOH. Boil the solution. Colour is not discharged, blue colour remains. Add 1 cc of 0.2% glucose or fructose solution and boil.

Result : The solution is **decolourised** due to formation of leuco-methylene blue, the reduction product of methylene blue.

Experiment (2) : **Reduction of alkaline ferricyanide.**

Procedure : In a test tube take 3 cc of 1% potassium ferricyanide solution and add 1 cc of 40% NaOH solution. Boil the solution. Add 0.2% glucose solution to the hot solution drop by drop and keep on boiling.

Result : The yellow colour of the ferricyanide begins to fade and finally **decolourise**.

Experiment (3) : **Tommer's test. Reduction of alkaline copper sulphate.**

Procedure : In a test tube take 2 cc of 0.5% copper sulphate solution, then add 2 cc of 0.2% glucose solution and mix. Add 2 cc of 40% NaOH solution. A clear blue solution is obtained. Glucose acts as a solvent for cupric hydroxide $Cu(OH)_2$ and prevents its precipitation. Boil.

Result : **Yellow or red precipitate** of Cu_2O is formed due to the reduction of $CuSO_4$.

$$(OH)_2 - O - Cu_2O + 2H_2O.$$

Experiment (4) : **Fehling's test.**

Procedure : Take 5 cc of Fehling's solution and boil. There is no change of colour on the formation of precipitate. (in case of colour change and precipitate formation reject the solution). Add 1 cc of glucose solution and boil again.

Result : Colour changes with the formation of **yellow** or **brick-red precipitate** of Cu_2O.

Experiment (5) : **Benedict's test.**

Procedure : In a test tube 5 cc of Benedict's reagent, then add 0.5 cc of glucose solution and heat to boiling. Boil for 2 minutes. Cool the solution under tap water.

Result : **Green, yellow or red precipitate** of Cu_2O is formed.

Experiment (6) : **Picric acid test.**

Procedure : In a test tube take 3 cc of 2% glucose solution, then add 1 cc of picric acid saturated solution and then add 1 cc of 40% NaOH.

Result : Picric acid is reduced to picramic acid with the formation of **red colour**.

$$C_6H_2OH(NO_2)_3 + 3H_2 \rightarrow C_6H_2OH.NH_2(NO_2)_2 + 2H_2O$$

Experiment (7) : **Nylander's test.**

Procedure : In a test tube take 5 cc of 2% glucose solution, add 0.5 cc of Nylander's reagent and heat to boiling and keep on boiling for 2 minutes.

Result : The solution becomes **dark black**, as bismuth sub-nitrate is reduced to bismuth.

$$Bi(OH)_2NO_3 + KOH \rightarrow Bi(OH)_3 + KNO_3$$

$$2Bi(OH)_3 + 3O \rightarrow 2Bi + 3H_2O$$

Experiment (8) : **Rapid furfural test.**

Procedure : Take 1 cc of 2% fructose solution, add 6 drops at α-naphthol, then add 5 cc of conc. HCl in a test tube and boil.

Result : As the mixture begins to boil, **deep purple colour** appears.

water. Now, the test tube is cleaned properly

BIOCHEMICAL TESTS

Some important test on Carbohydrates, Proteins, Fats and Enzymes have been described here.

1. Test on Carbohydrates

Carbohydrates are abundantly found in the plants and as glycogen in the animals. In animals they are found in free stored state as glycogen or in combination with proteins as glycoprotein. The name carbohydrate is given because they are composed of **carbon**, **hydrogen** and **oxygen atoms**. H_2 and O_2 are found in the same proportions as in water (H_2O). Chemically carbohydrates are aldehyde and ketone derivatives of alcohols (aldoses and ketoses). In general, carbohydrates are white solids, sparingly soluble in organic liquids, except for certain polysaccharides, soluble in water. Many carbohydrates are of low molecular weight and having sweet taste. Carbohydrates are classified into 3 groups :

(1) **Monosaccharides** or simple sugars ($C_6H_{12}O_6$),
(2) **Di-** and **tri-saccharides** or compound sugars,
(3) **Polysaccharides**.

A. MONOSACCHARIDES

Monosaccharides or simple sugars occur abundantly in nature in the form of glucose and fructose. They occur in white crystalline form easily soluble in water and hot alcohol and practically insoluble in organic solvents like absolute alcohol, ether and acetone, etc. They are optically active and being aldehydes and ketones show common reactions.

In alkaline solution all the monosaccharides and many disaccharides behave as reducing agents and are easily oxidised by various reagents as silver and copper, etc. Most of the quantitative analysis for sugars depend upon the measurement of the reduction of Cu^{++} to Cu^{+} by alkaline sugar solutions.

B. EXPERIMENTS WITH GLUCOSE AND FRUCTOSE

Make 0.2% and 2% solutions of the Dextrose-D or Fructose and perform the following experiments in order to identify the reducing action of glucose and fructose :