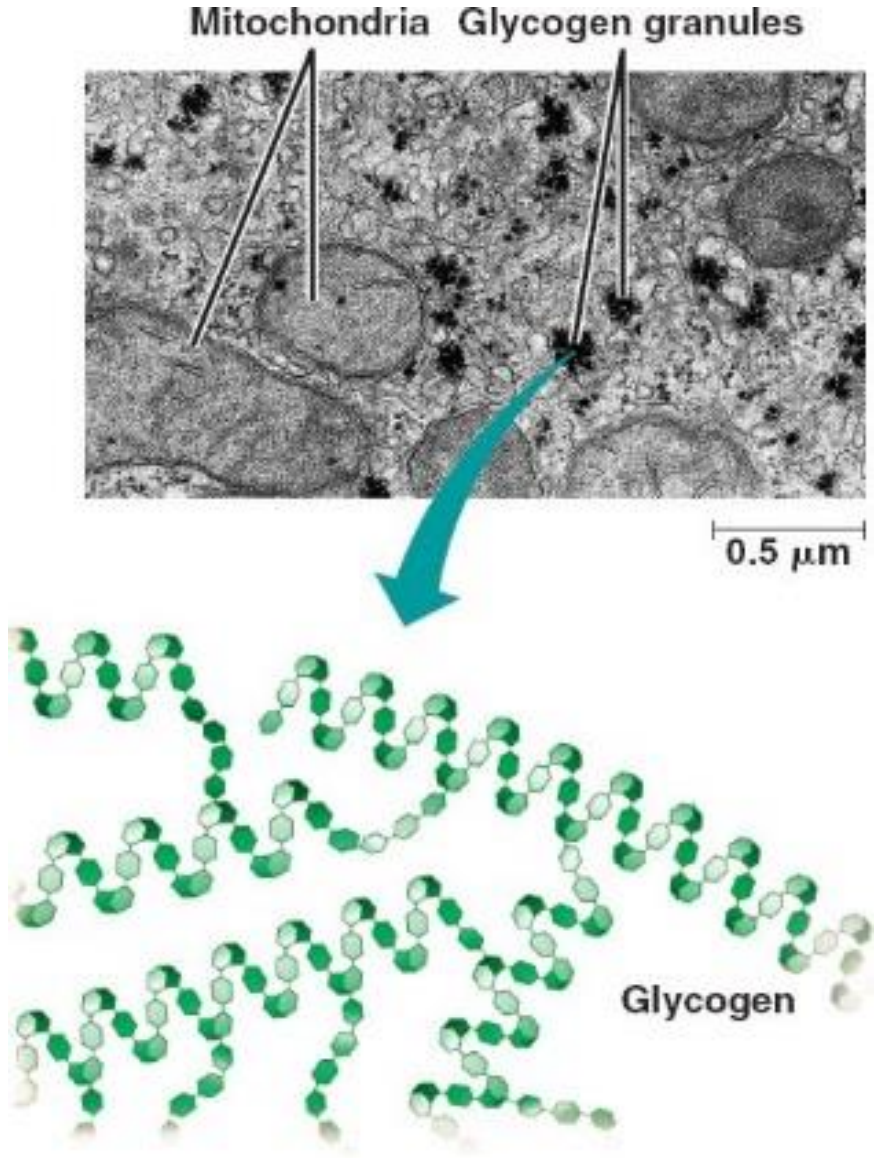
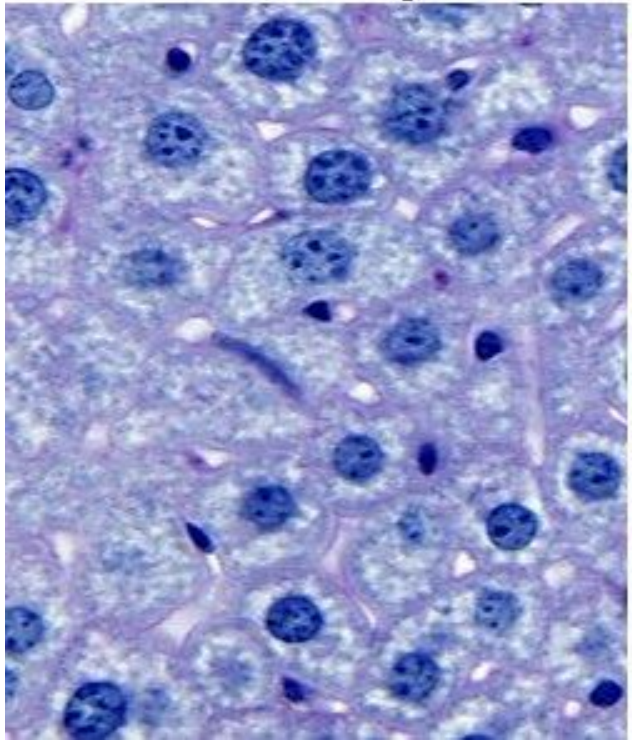
Experiment 1

Isolation of Glycogen from rat Liver

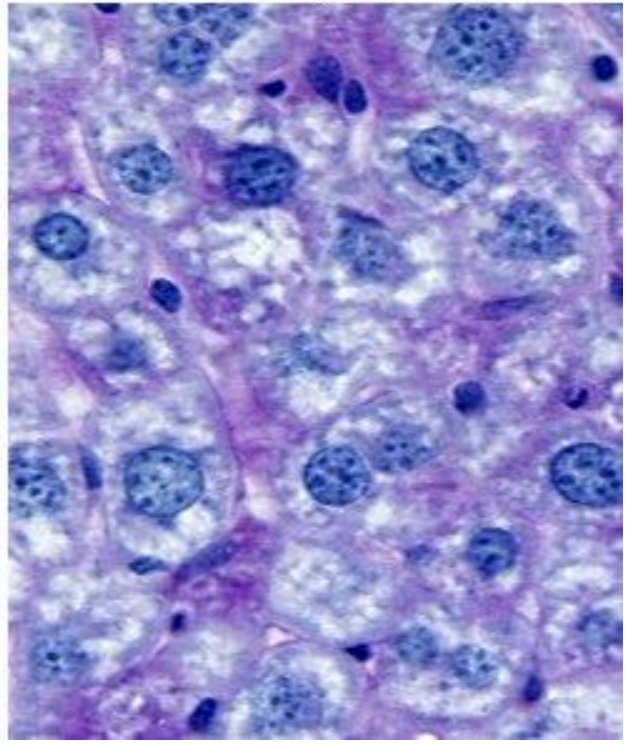
Amal Alamri



Fasted overnight



Two hours post-feeding



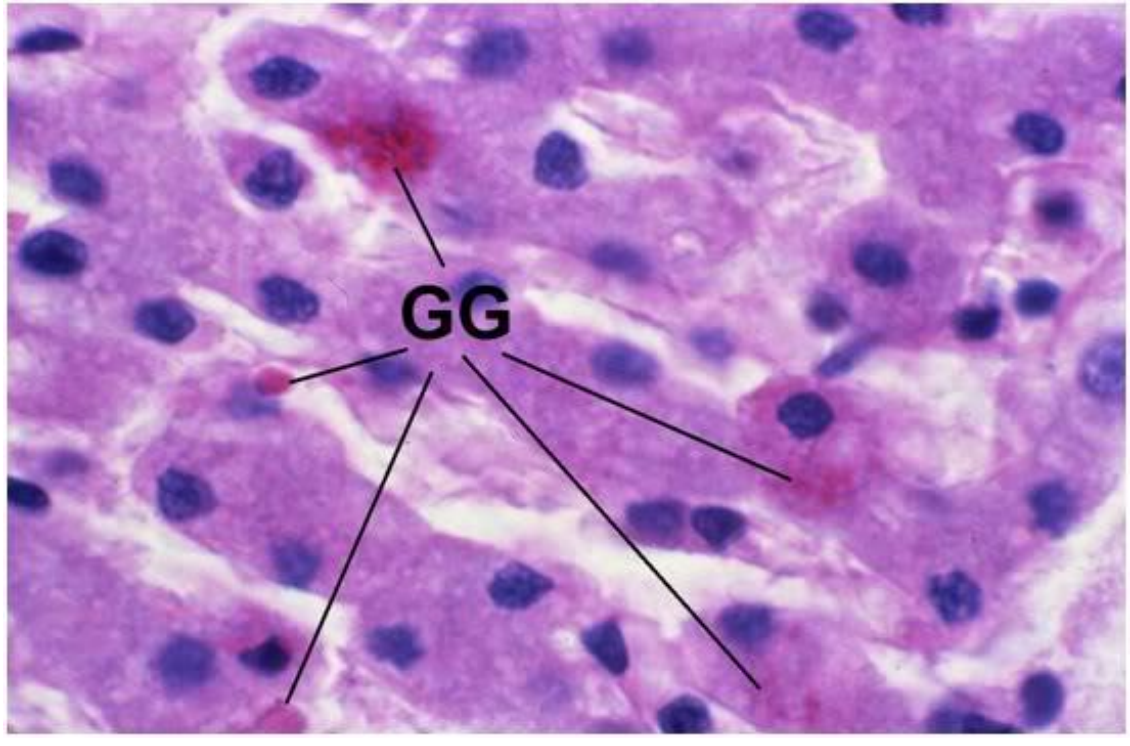


Figure 35: *FIG-2, Liver, PAS, 100x. Note the presence of a few scattered glycogen granules (**GG**).*

Objective

- To illustrate the method for isolating glycogen.

principle :

De proteinized homogenate of rat liver , then the glycogen is precipitated out by ethanol

Materials and Equipments

- Trichloroacetic acid 5% and 10% TCA
- Ethanol (95% v/v)
- Diethyl ether
- Sodium chloride
- Liver (of well fed rat or other animal)
- Washed and dried sand
- Ice
- *Mortar and pestle*
- *Refrigerated centrifuge*
- *Water bath at 37ºC.*
- *Glass rods*
- *Two 20 ml beakers*
- *One 100 ml beaker*
- *100 ml graduated cylinder*
- *50 ml graduated cylinder*

Caution! TCA causes severe burns; wash accidental spills on skin with plenty of running tap water for a minute.

Sample Preparing

- The condition of the animal from which the liver is taken is important because the yield varies according to whether the animal is fed, fasted, ill etc. Therefore for a good yield the animal should be well fed before the liver is removed, the sample should be kept cold and the pH lowered with TCA.

- in initial stage of isolation glycogen the Temperature should be decrease (Cold) and the PH low : because these condition important to inhibit the enzymatic hydrolysis of glycogen (to protect the glycogen structure and getting good yield)

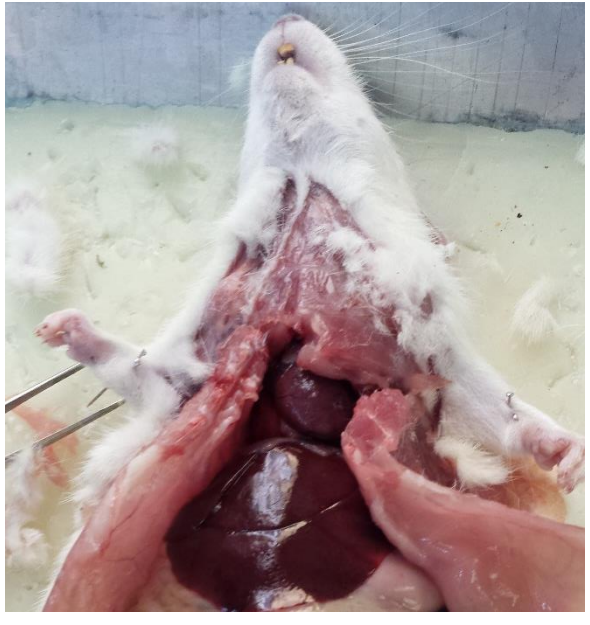
Sample Preparing

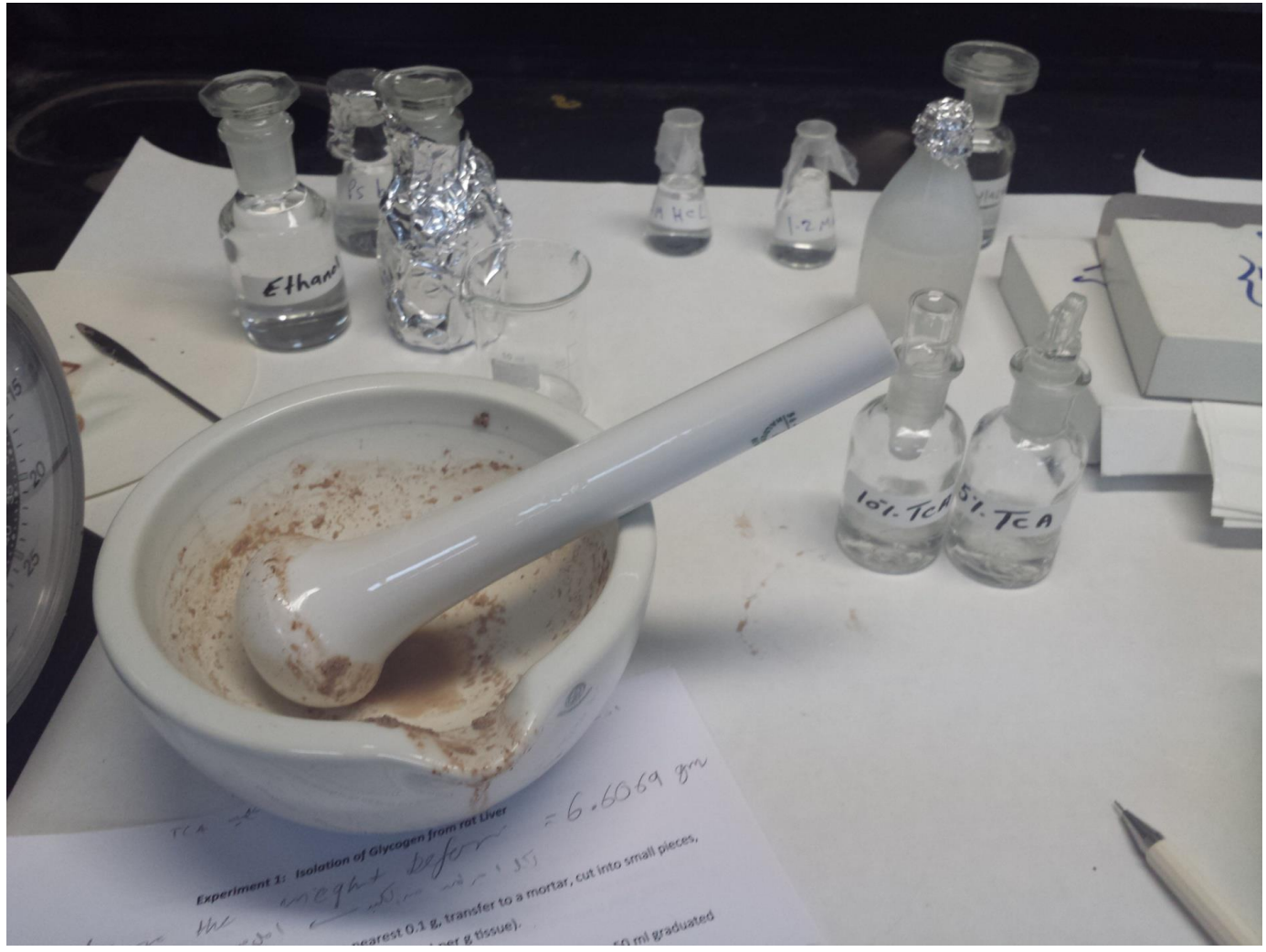
- When liver is ground up with tri chloroacetic acid (TCA), the *larger molecules such as proteins and nucleic acids are precipitated while glycogen remains in solution with sugars and other water–soluble compounds*.

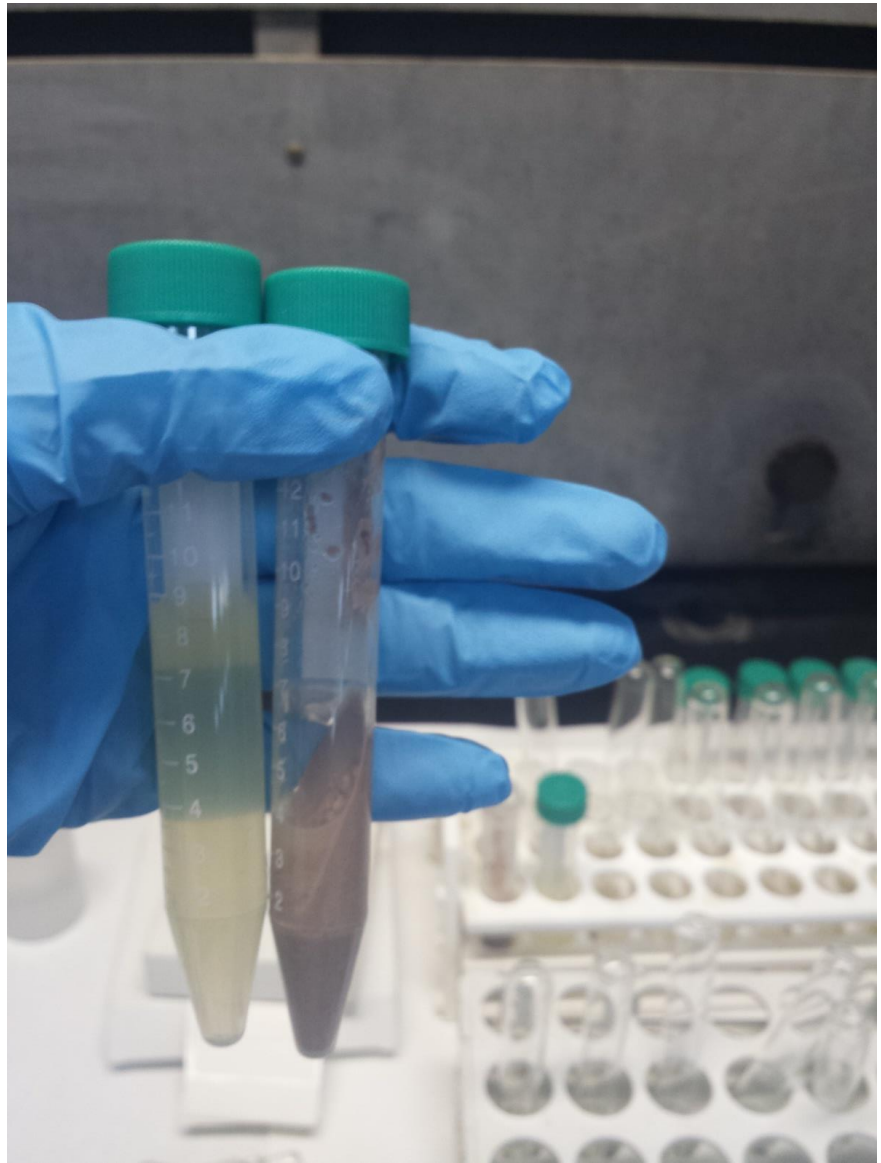
- The glycogen can then be separated from the other compounds by precipitation with aqueous alcohol in which it is less soluble.

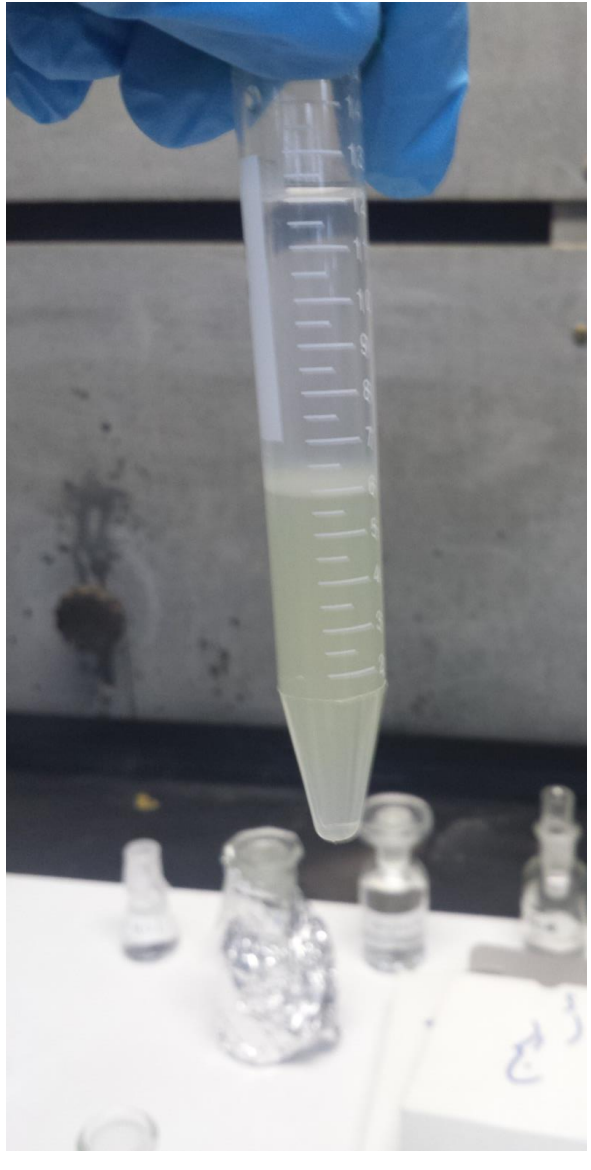
Procedure

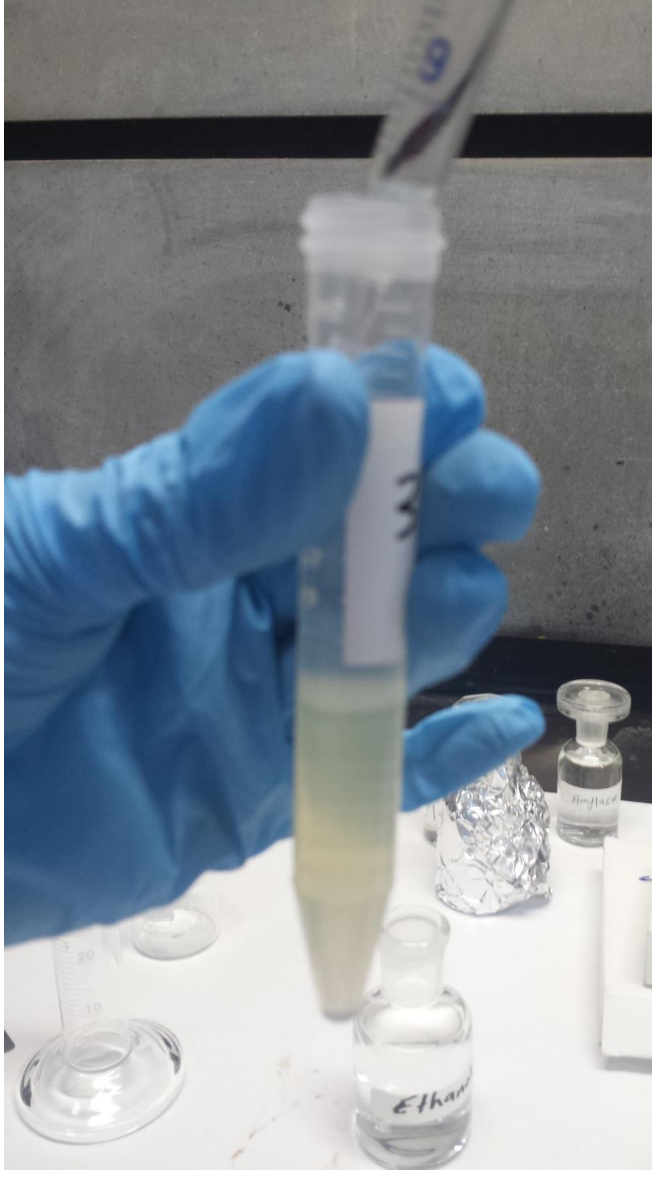
- 1. Weigh about 5.0 g of cold liver**, cut into small pieces, grind with about 0.5 g of clean cold sand and 10%TCA (1 ml per g tissue).**
- 2. Centrifuge homogenate at 3,000 rpm for 5min at 4oC. Pour off supernatant into a 50 ml graduated cylinder.
- 3. Rinse out mortar with 5% TCA (using same volume as for 10% TCA already used). Add this rinsing fluid to the centrifuge tubes containing residue from first centrifugation. Stir up residue and re-centrifuge for another 5 min. at 3,000 rpm. Discard pellet. Add supernatant to that already collected.




Ethanol
10% TCA
5% TCA
Experiment 1: Isolation of Glycogen from rat Liver
= 6.6069 gm

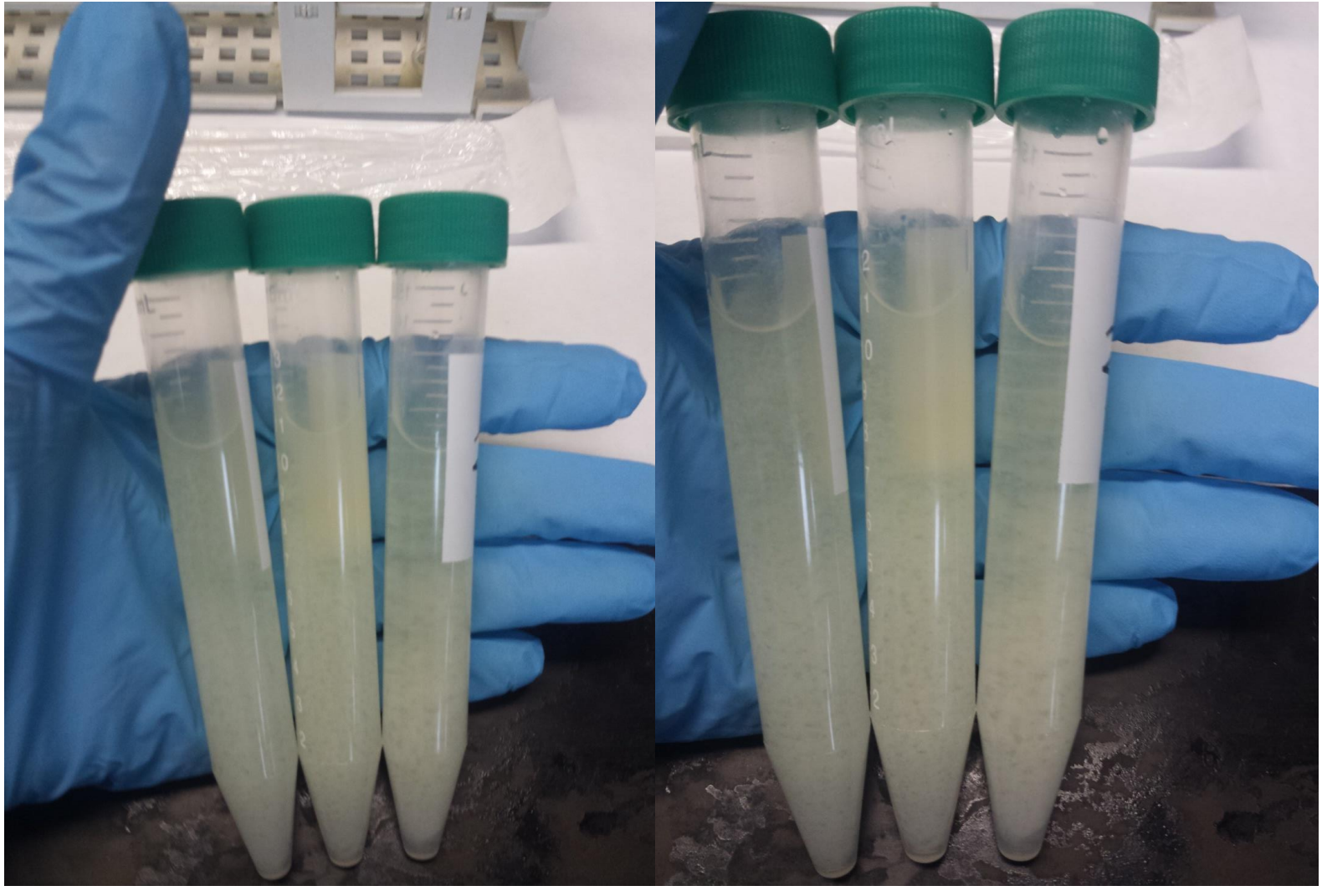


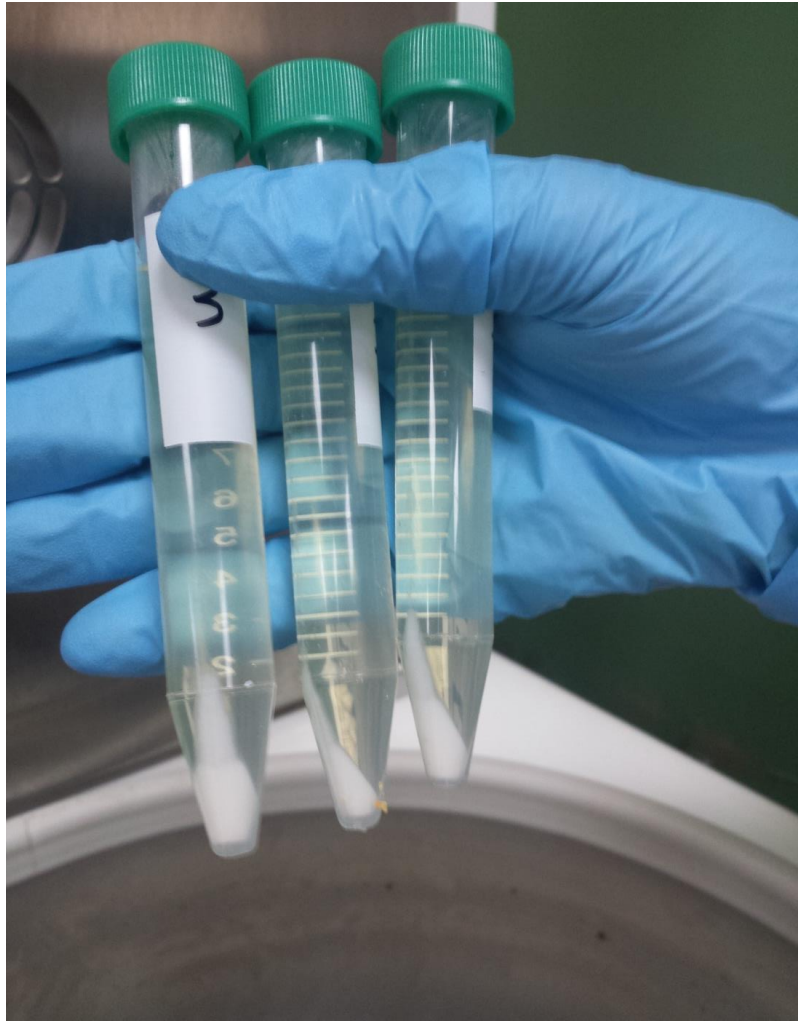




- 4. Record total volume; add twice this volume of 95% ethanol, slowly with stirring, to supernatant. Allow to stand while precipitate settles. If it does not, add a little NaCl and warm cylinder in water bath at 37º C.
- 5. Centrifuge suspension at 3,000 rpm for 3 min. Discard supernatant. Dissolve pellet in centrifuge tubes in 5 ml water and re-precipitate by adding 10 ml of 95% ethanol. Re-centrifuge and discard supernatant.
- 6. Stir up pellet with 3 ml 95% ethanol, re-centrifuge and discard

supernatant. Now add 3 ml diethyl ether, stir up pellet, re-centrifuge and discard supernatant. This final pellet contains glycogen from the liver. Air -dry the glycogen in the tube and weigh it.

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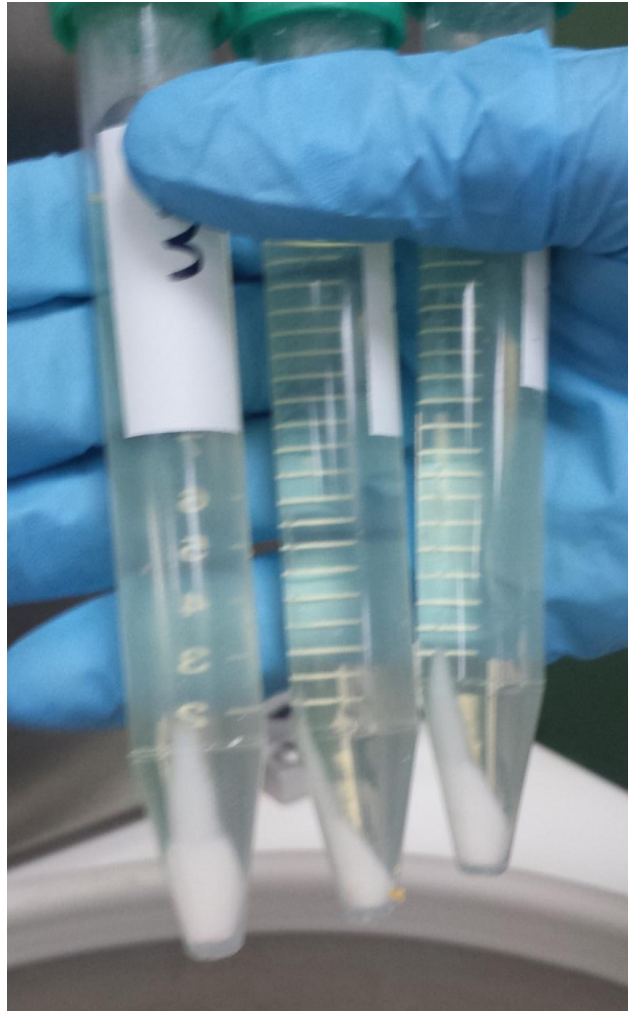
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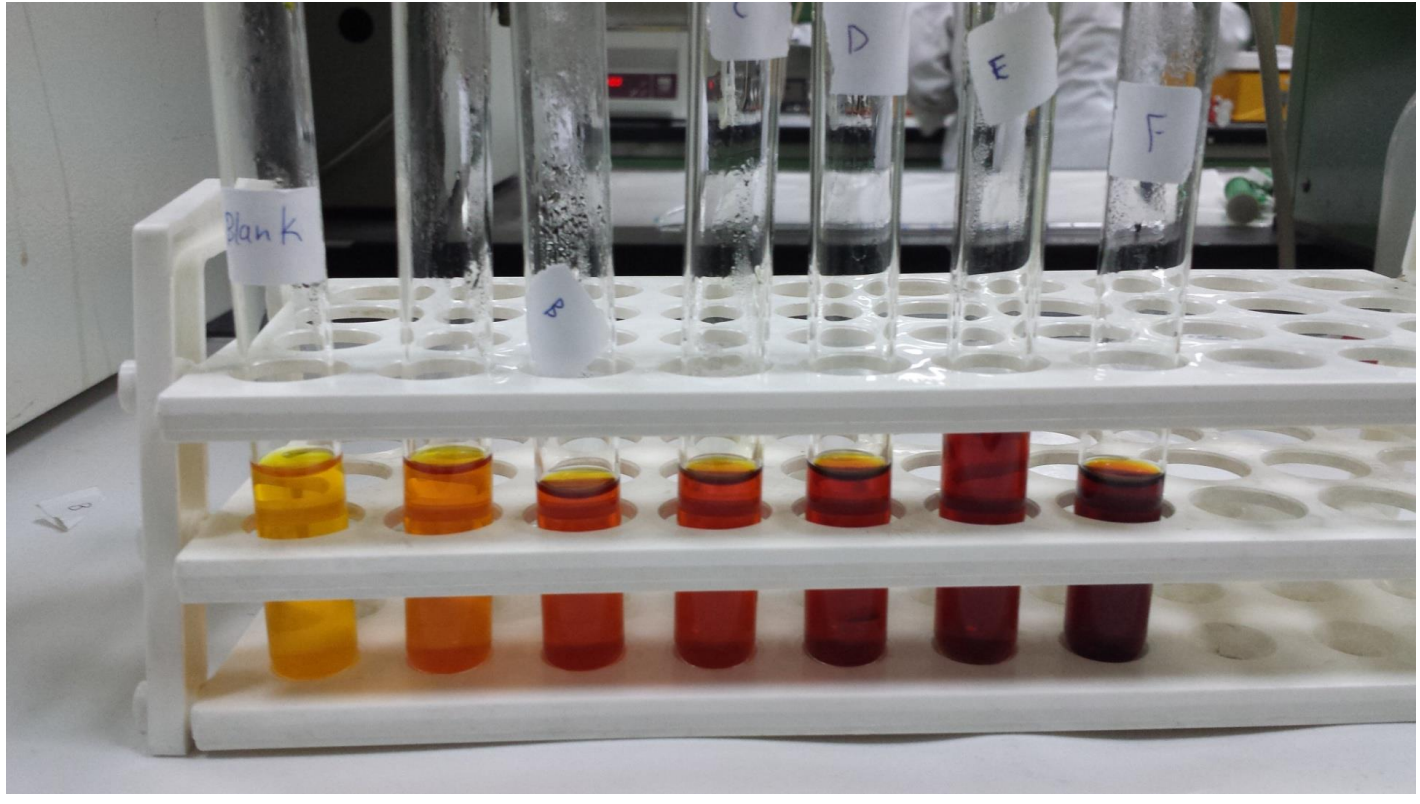
Experiment 2 :Enzymatic hydrolysis of glycogen and determination of glucose



Results

Glycogen content (g) =

centrifuge tube that contain pellet - empty Centrifuge tube

- Record total yield and glycogen content per 100 g liver.

Example:

Suppose that the liver weight is 10 g and the glycogen yield was 1.5 g

Then the glycogen content per 100 g is

1.5 g ⟶ 10 g liver

x ⟶ 100 g liver

= 1.5 X 100 / 10

= 15 g /100 g liver

Discussion

- Record total Glycogen content (g) =

- Record total yield and glycogen content per 100 g liver.

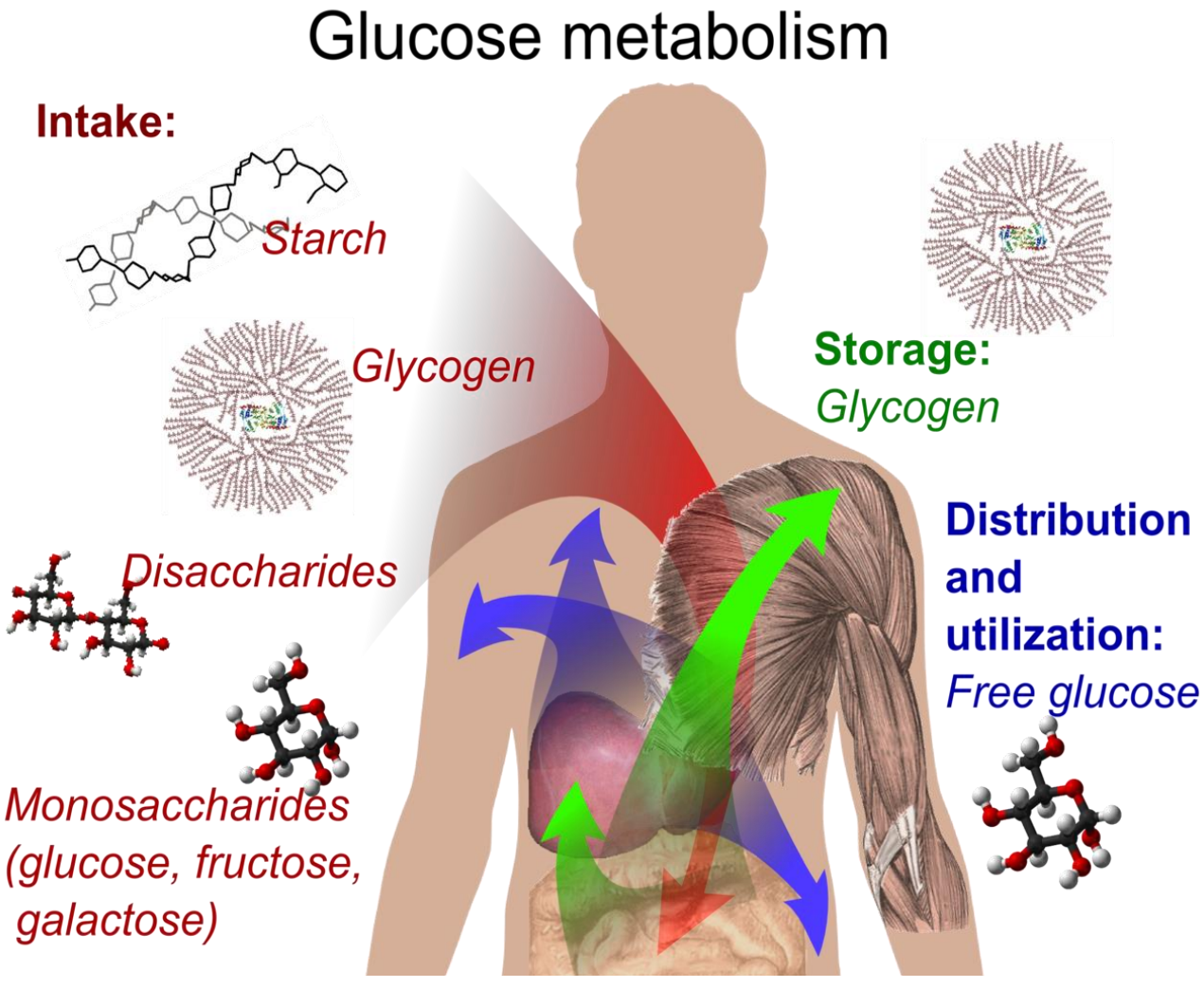
Question

Why are time, temperature and pH important in the initial stages of the isolation of glycogen, but not in the latter stages?

Experiment 2

Enzymatic hydrolysis of glycogen and determination of glucose

Amal Alamri



hydrolysis of glycogen

- The structure of the glycogen molecule is fan-like; with long chains of glucose residues linked by 1, 4-glycosidic bonds, with 1, 6- links at the branch points.
- *the whole glycogen molecule has only one free reducing end, where the C1 of a glucose residue is free (exposed). Thus the glycogen molecule is non-reducing*.
- Hydrolysis converts glycogen from a non-reducing substance into reducing substances.

- Hydrolysis with acid results in splitting of all glycosidic bonds *giving only glucose* molecules as the product.
- Enzymes are more specific in the bond type they split. Thus salivary amylase (α-amylase) will randomly split only 1, 4-glycosidic bonds and produce a mixture of products consisting *of glucose, maltose and maltotriose molecules*.

The increase in the number of reducing groups is determined using 3, 5-dinitrosalicylic acid (DNS) in alkaline solution.

Enzymatic Hydrolysis of glycogen by α-amylases

- The amylases of animal origin are all α-amylases and in the digestive system are found in saliva and in pancreatic juice.
- α-amylases catalyze the rapid, random hydrolysis of internal α-1, 4 bonds. They do not hydrolyze α-1, 6 linkages, regardless of molecular size nor do they hydrolyze maltose.
- Further action of α-amylase decreases the molecular weight of these dextrins yielding oligosaccharides. The final degradation products of the action of α-amylase on glycogen are glucose, maltose and isomaltose.

Enzymatic Hydrolysis of glycogen by β-amylases

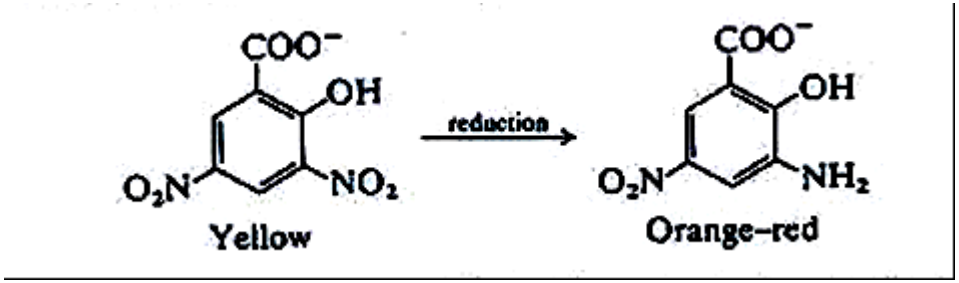
- β-amylases is widely distributed in plants and microorganisms. It catalyze the successive hydrolysis of the second α-1, 4 glycosidic bond from the free nonreducing ands of glucose chains, releasing maltose units.
- But β-amylases **do not hydrolyze α-1, 6 bonds**, nor do they hydrolyze α-1, 4 bonds of glucose chains beyond an α-1, 6 branch residues.
- the final products of the action of β-amylase on glycogen are maltose and the remaining limit dextrin.

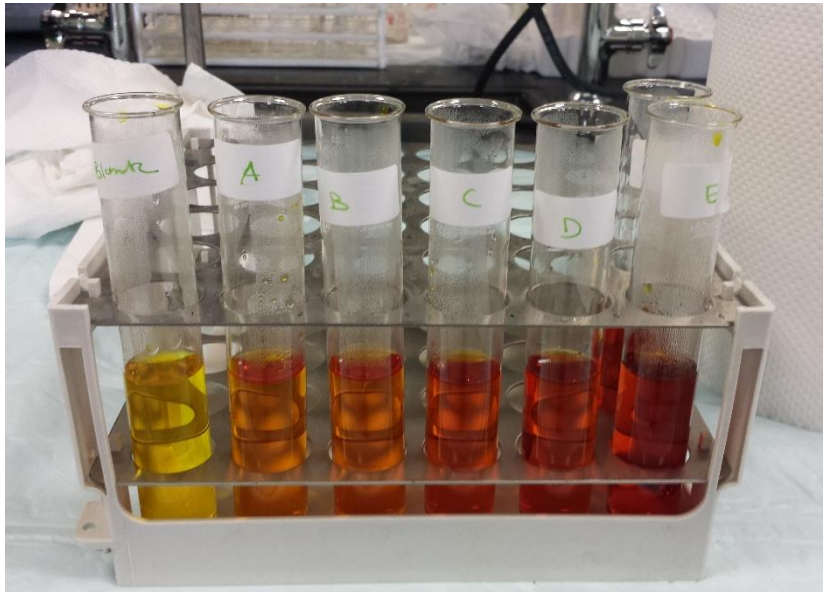
Objective

- To examine the polysaccharide nature of glycogen and show that hydrolysis increases the number of reducing groups.
- To estimate the amount of glucose produced by hydrolysis of glycogen using DNS reagent.

Principle

In alkaline solution it is reduced to 3-amino-5- nitro salicylic acid, which is orange-red. Absorbance is determined at 540 nm.





Materials and Equipments

- Glycogen isolated in the previous experiment
- Sodium dihydrogen phosphate (NaH_2PO_4)
- Sodium hydroxide
- Sodium chloride
- Sodium potassium tartrate
- 3,5-Dinitrosalicyclic acid (DNS)
- HCl
- Boiling water bath
- Spectrophotometer
- Small beaker
- Big test tubes (25 ml)
- Glass cuvettes

Discussion:

Comment on the results and the concentration of glucose yield.

Question :

What would be the effect of substituting β-amylase for α-amylase?