Experiment 10: Isolation of Glycogen from Liver

Procedure:

1. You have provided with the Liver samples which was stored in 0.9% NaCl as preservative. The sample weights the liver and record the weight

2- Quickly transfer it to a mortar, cut it into small pieces, and grind with about 0.5 g of cold sand and **10% TCA (1 ml per g tissue).**

How many ml of 10%TCA you will add to your sample?

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3. Centrifuge homogenate at 3,000 rpm for 5min at 40C.

4. Pour off supernatant into ml graduated cylinder.

5. Rinse out mortar with **5% TCA** (using same volume as for 10% TCA already used).

6. Add this rinsing fluid to the centrifuge tubes containing residue from first centrifugation.

7. Stir up residue and re-centrifuge for another 5 min. at 3,000 rpm.

8. Discard pellet. Add supernatant to that already collected.

9. Record total volume; add the double of the supernatant 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 37° C.

How many ml of supernatant you obtained and how many ml of 95% ethanol you will add to your sample?

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10. Centrifuge suspension at 3,000 rpm for 3 min. Discard supernatant.

11. Now add 3 ml diethyl ether, stir up pellet, re-centrifuge and discard supernatant. This final pellet contains glycogen from the liver.

12. Air -dry the glycogen in the tube and weigh it.

Results:

Weigh the centrifuge tube that contains the glycogen =........................ gm
Weigh the empty centrifuge used in this experiment. =........................ gm
So Glycogen content (g) =
Centrifuge tube that contain pellet -empty Centrifuge tube
....
....
....

Record total glycogen yield in 100 g liver.

....
....
....

Experiment 11:
Enzymatic hydrolysis of glycogen and determination of glucose

A-Enzymatic hydrolysis of glycogen

Procedure:

1. Dissolve the glycogen you have precipitated in Phosphate buffer. And label it as glycogen solution.

Each 0.032 g glycogen Dissolve in 4 ml phosphate buffer

How many ml of phosphate buffer you will add to your sample?

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2- Label 9 tubes 1 -9.

Tube No.	PS Buffer (ml)	Glycogen Solution (ml)
Blank	0.4	-
1	-	0.4
2	-	0.4
3	-	0.4
4	-	0.4
5	-	0.4
6	-	0.4
7	-	0.4
8		

Addition I: Table (1)

Tube No.	PS Buffer (ml)	Glycogen Solution (ml)	Dilute amylase (ml)	2M HCl (ml)	Time of Hydrolysis (min)	1.2.M NaOH (ml)	DNS Reagent (ml)	Water (ml)
Blank	0.4	-	0.6	-	30	-	1	8
1	-	0.4	0.6	-	0	-	1	8
2	-	0.4	0.6	-	2	-	1	8
3	-	0.4	0.6	-	4	-	1	8
4	-	0.4	0.6	-	6	-	1	8
5	-	0.4	0.6	-	8	-	1	8
6	-	0.4	0.6	-	10	-	1	8
7	-	0.4	0.6	-	30	-	1	8
8			-	0.6	30	1	1	7

Addition table II: Table (2)

Time table: Table (3)

Tube No.	**START BY 0.6 ml α-amaylase**	2 M HCl(0.6ml)	Total Time of Hydrolysis (min)	**STOP** BY **1ml** DNS Reagent	Water (ml)
Blank	0	**-**	**30**	**30**	**8**
1	1	**-**	**0**	**1**	**8**
2	2	**-**	**2**	**4**	**8**
3	3	**-**	**4**	**7**	**8**
4	4	**-**	**6**	**10**	**8**
5	5	**-**	**8**	**13**	**8**
6	6	**-**	**10**	**26**	**8**
7	7	**-**	**30**	**37**	**8**
8	8		**30**	**38**	

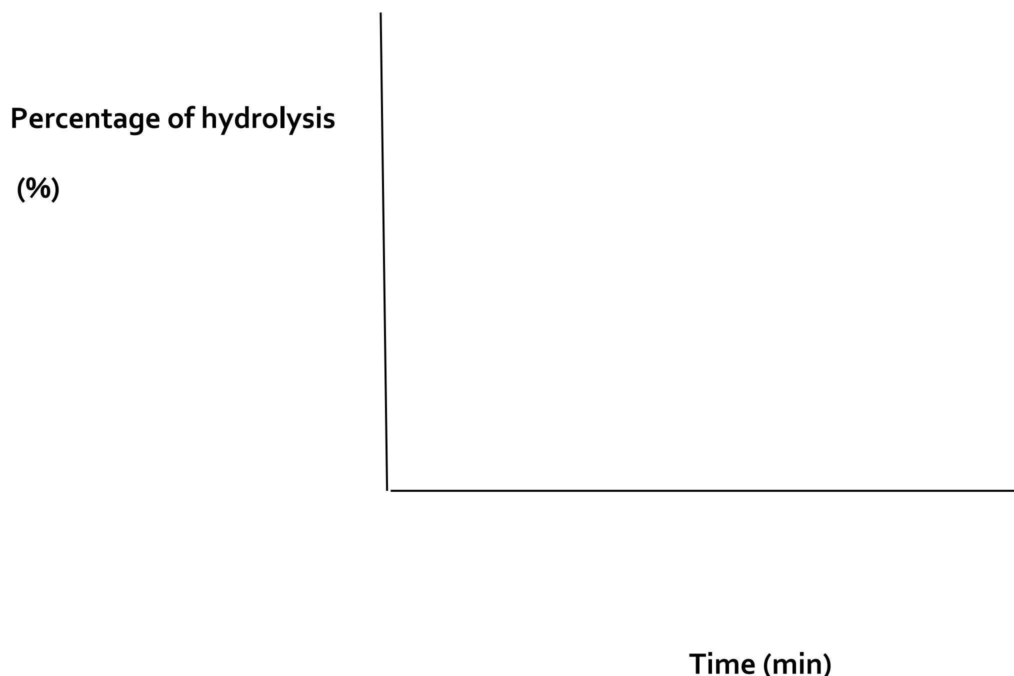
Results:

- **Tube 8 contained the total glucose yield from complete hydrolysis of glycogen. Taking this as 100% conversion of glycogen to glucose, plot the percentage hydrolysis against time.**

Tube No.	Time of hydrolysis(min)	Absorbance (540 nm)	Percentage hydrolysis
Blank			
1			
2			
3			
4			
5			
6			
7			
8			100

Percentage hydrolysis % = (Absorbance of n tube ÷ Absorbance of tube 8) x100

- **Plot the curve show the relationship between percentage of hydrolysis and time**



Time (min)

B- Determination of glucose yield from glycogen hydrolysis, by preparation of Calibration Curve or Reducing Sugars.

Method:

1- Prepare 7 test tubes in the following manner , table (1):

Tube	Water (ml)	0.005M Standard reducing sugars(ml)	Acetate buffer (ml)	DNS reagent .(ml)
Blank	2.0	-	1.0	2.0
A	1.8	0.2	1.0	2.0
B	1.6	0.4	1.0	2.0
C	1.2	0.8	1.0	2.0
D	1.0	1.0	1.0	2.0
E	0.5	1.5	1.0	2.0
F	-	2.0	1.0	2.0
Sample	-	-	-	-

2-Mix each tube properly then, cover each tube with aluminium foil and place in a boiling water bath for 5 min .

3-Remove the tubes from the water bath , cool under tap water , then add 20ml of water to each tube and mix properly .

4-Measure the absorbance of each tube against the blank at 540 nm, then record the absorbance in the following table (2) .

5 -Construct the calibration curve by plotting the absorbance at 540nm against the Concentration of reducing sugars in micro Molar.

Tube	Absorbance 540nm	Concentration of reducing sugars M.	Concentration of reducing sugars µM.
A			
B			
C			
D			
E			
F			
Sample			

Table (2).