Lab sheet 3

Isolation of Glycogen

Method:

1- Weigh about 5.0 g of cold liver quickly to the nearest 0.1 g, transfer to a mortar, 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 4 ^{o}C. 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.

4- Record total volume; add the equal 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^{o} 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.

7- Air-dry the glycogen in the tube and weight it.

8- Dissolve 32 mg glycogen in 4 ml phosphate buffer/NaCl.

Results:

Empty test tube weight:	_____
The weight of the glycogen + test tube:	_____
The weight of the glycogen (g):	(The weight of the glycogen + test tube) – (The weight of the empty test tube) =

Calculations:

a) The volume of <u>phosphate buffer</u> needed:

_____
_____
_____
_____

b) Record total yield and glycogen content per 100 g liver.

_____
_____
_____
_____