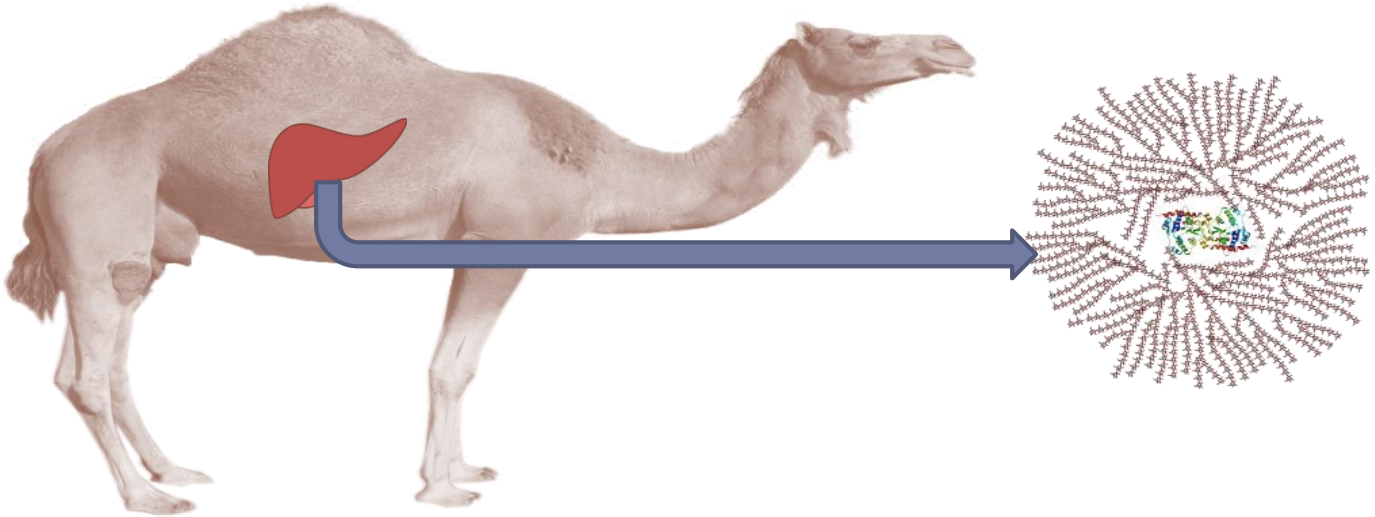


Isolation of Glycogen from Camel's liver

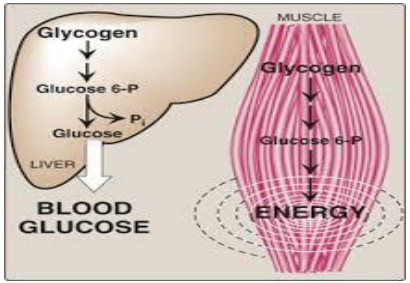
BCH 447

Objective:

- To illustrate the method for isolating glycogen.

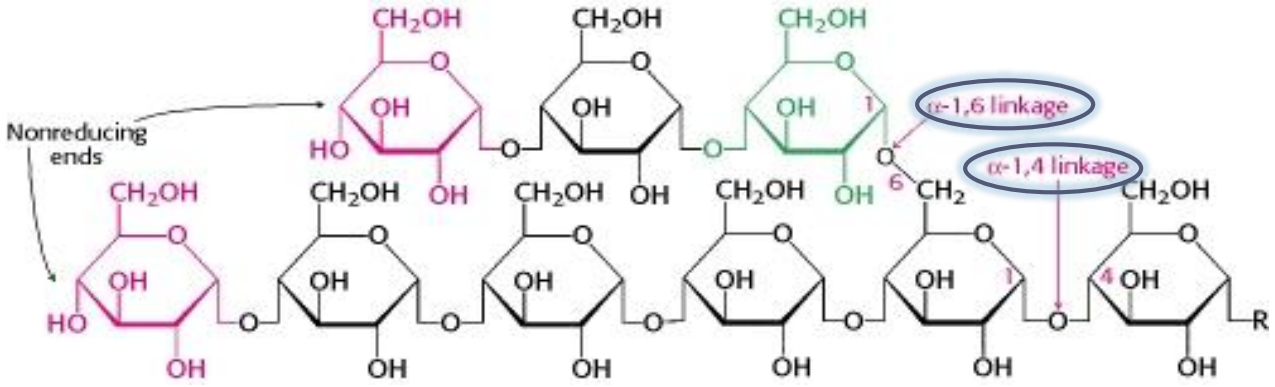
Glycogen

- Glycogen is the main polysaccharide energy reserve in animals which is analogous to the starch in plants.
- It is the storage form of glucose.
- It is synthesized and stored mainly in **liver and muscles**.
- Its concentration **in the liver is higher** than in muscles.



Structure of glycogen

- It is a very large, branched polymer of glucose residues.
- Various samples of glycogen have been measured at 1,700-600,000 units of glucose.



Why does the body store glycogen?

- The breakdown of glycogen and release of glucose increase the amount of glucose that is available between meals.
- Hence, glycogen **serves as a buffer** to maintain blood-glucose levels.
- Maintaining blood-glucose levels is especially important because glucose is virtually the only fuel used by the *brain*, except during prolonged starvation.
- Moreover, the glucose from glycogen is readily metabolized and is therefore a good source of energy for sudden activity.

Principle of the experiment:

Grinding with TCA and sand

- Trichloracetic acid (TCA)is used to precipitate large molecules by **denaturation**
- Sand helps in the grinding process

Centrifugation

- Proteins and nucleic acids are precipitated
- Glycogen remain in the supernatant with other sugars and water soluble substances

Alcohol Precipitation

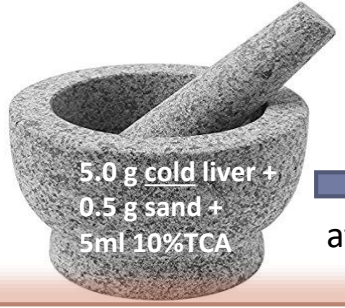
- Glycogen is separated by precipitation with alcohol, **polysaccharides are less soluble than sugars in aqueous alcohol.**

Procedure:

CAUTION

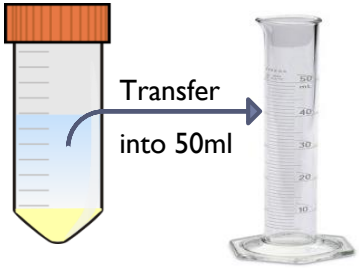
TCA causes severe burns; wash accidental spills on skin with plenty of running tap water.

1



Centrifuge

at 3,000 rpm for 5min



Why cold? because low temp. inhibit the enzymatic hydrolysis of glycogen

2

Centrifuge

at 3,000 rpm for 5min

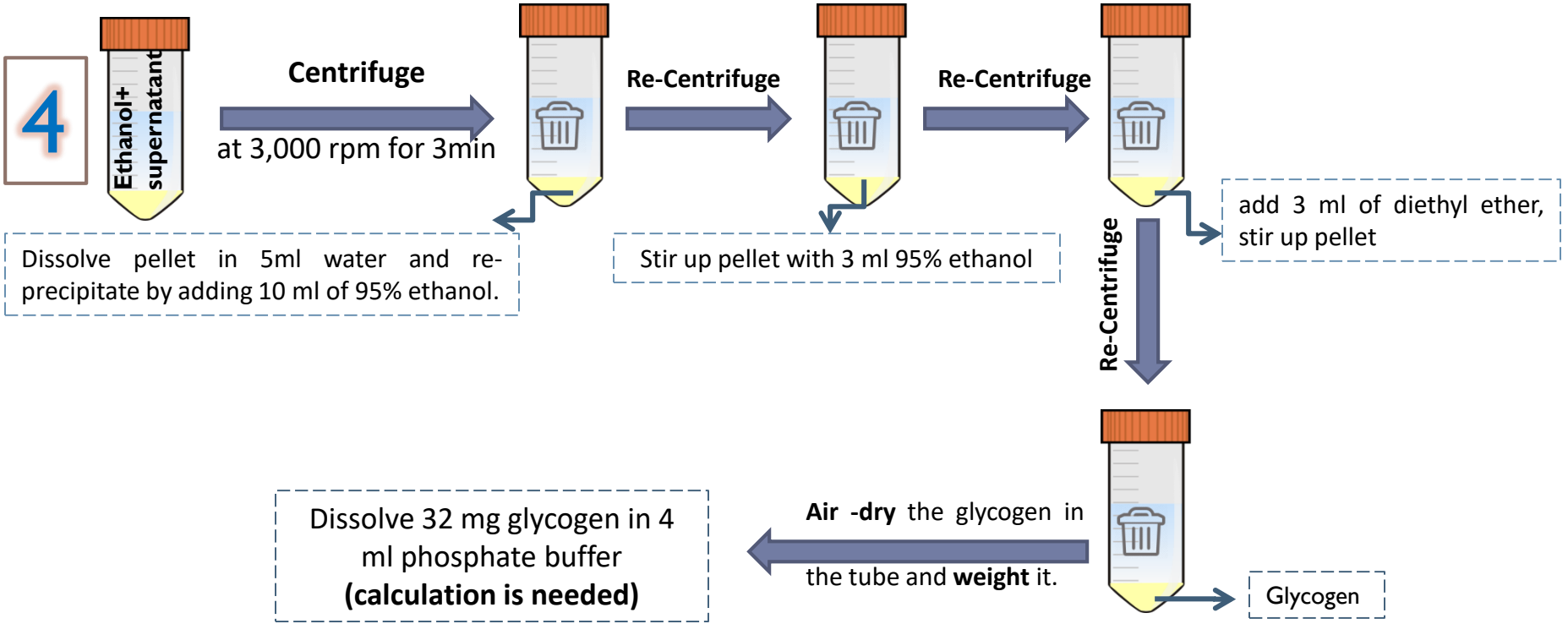
3

-Record total volume.

-Add equal volume of 95% ethanol, slowly with stirring.

-Allow to stand while precipitate settles.

Procedure:


4
Ethanol+ supernatant
Centrifuge
at 3,000 rpm for 3min
Re-Centrifuge
Re-Centrifuge
add 3 ml of diethyl ether, stir up pellet
Re-Centrifuge
Stir up pellet with 3 ml 95% ethanol
Dissolve pellet in 5ml water and re-precipitate by adding 10 ml of 95% ethanol.
Glycogen
Air -dry the glycogen in the tube and weight it.
Dissolve 32 mg glycogen in 4 ml phosphate buffer
(calculation is needed)

Calculations:

Glycogen content (g) =  - 

How to make glycogen solution?

Dissolve glycogen pellet in **phosphate buffer** (**32mg glycogen in 4 ml phosphate buffer**)

- Example:

I have 3.52 g glycogen ??

A- 3.52g X 1000 = 3520 mg

B- 32mg → 4 ml

3520mg →??? ml

Results:

Record total yield and glycogen content per 100 g liver.

Example:

Liver weight 5 g - The glycogen content 1.5 g

** the glycogen yield was 1.5 g

** the glycogen content per 100 g is

1.5 g → 5 g liver

??g → 100 g liver

= 1.5 X 100 / 5 = 30g /100 g liver

factors affecting glycogen yield:

-Food state: Fed animals get more glycogen yield

-Stress: Any stress for animal will lead to glycogen consumption .