The assay of tissue glycogen

Principle:

Glycogen is released from the tissue by heating with strong alkali and precipitated on the addition of ethanol. Sodium sulphate is added as a co precipitant to give a quantitative yield of glycogen.
The polysaccharide is then hydrolyzed in acid and the glucose released is estimated.

Materials:

1. Heart, liver, and muscle from a freshly killed rat.
2. potassium hydroxide (300 g/l)
3. Calibrated centrifuge tubes (10 ml). 30
4. Boiling water bath. 24
5. Saturated Na_2 S04. 20 ml
6. Ethanol (95% v/v). 250 ml
7. Volumetric flasks (100 ml). 24
8. Test tubes calibrated at 10 ml. 100 ml
9. HCl (1.2 mol/l.). 100 ml
10. Marbles.
11. Phenol red indicator solution. 12 ml
12. NaOH (0.5 mol/l). 250 ml
13. Reagents for the estimation of glucose (Experiment 1).

Procedure:

Isolation of glycogen:-

Accurately weigh the complete heart and muscle and about 1.5 g of liver. Place the tissues into a calibrated centrifuge tube containing 2 ml of KOH (300 g/l) and heat in a boiling water bath for 20 min with occasional shaking. Cool the tubes in ice, add 0.2 ml of saturated Na_2 SO_4, and mix thoroughly. Precipitate the glycogen by adding 5

ml of ethanol (95% v/v), stand on ice for 5 min, and remove the precipitate by centrifugation. Discard the supernatant and dissolve the precipitated glycogen in about 5 ml of water with gentle warming, then dilute with distilled water to the 10 ml calibration mark and mix thoroughly. In the case of the fed animals, transfer the liver sample quantitatively to a 100 ml volumetric flask and make up to the mark with water.

Hydrolysis and estimation of glycogen:-

Pipette duplicate 1 ml samples of the glycogen solutions into test tubes calibrated at 10 ml, add 1 ml of HCl (1.2 mol/l), place a marble on top of each tube, and heat in a boiling water bath for 2 h. At the end of this period, add 1 drop of phenol red indicator and neutralize carefully with NaOH (0.5 mol/l) until the indicator changes from yellow through orange to a pink color. Dilute to 5 ml with distilled water and determine the glucose content by the 3.5 dinitrosalisylic acid method (Experiment 1).Then use the standard curve you obtained to estimate the concentration of glucose per100 g sample.

Name: ***No.***

Experiment 6:

Results Sheet

Calculate the amount of glycogen in the liver sample, using the standard curve you plotted in experiment 1.