**Biology and Biochemistry Department**

**BIOC 312**

**Biochemistry II lab**

**Experiment #3**

**Sec 1**

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**Title**

**Glycogenolysis – Effect of nutritional state on tissue glycogen content**

**Objective:**

The major objectives of this lab is to see how starvation affects glycogen content in skeletal muscles and liver, as well as glycogenolysis rates in those tissues.

**Introduction:**

 Glycogenolysis is a process in which Glycogen is converted to 6-glucose phosphate in response to hormonal and neural signals. It primarily occurs in the liver and muscles. Glycogenolysis, in particular, is essential in the control and regulation of blood glucose levels.1 3

 In the glycogenolysis pathway there are three enzymes that control it, and these enzymes: First we have glycogen phosphorylase The addition of inorganic phosphate breaks the outer (α1🡪 4) glycosidic linkages, resulting in dextrins (shorter oligosaccharides), So by substituting a phosphoryl group for the (α1🡪 4)linkage, glycogen phosphorylase cleaves the bond linking a terminal glucose residue to a glycogen branch. The enzyme that breaks the (α1🡪6) branches is known as the debranching enzyme so this is the second enzyme. And finally the enzyme phosphoglucomutase converts glucose-1-phosphate to glucose-6-phosphate (which is frequently used in glycolysis)3

 Glucose is stored in mainly the liver and muscles as glycogen. It's utilized in tissues and is widely distributed. When rats are starved, the hormones epinephrine and glucagon are secreted, and the pathway's major purpose differs depending on whether the cells are hepatocytes or myocytes, with hepatocytes using it when blood glucose levels are low. Which leads to maintaining a normal glucose levels in the blood, On the contrary, we have muscles that are only affected by strenuous exercises and physical processes only, so that the muscles use this path only when exercising.5 2

 The main purpose of the Glycogenolysis pathway differs between liver cells and muscle cells due to two distinctions. The absence of glucagon receptor in muscle cells, which interacts with glucagon hormone in liver cells to stimulate glycogenolysis pathway when glucose blood concentration is low, is the first difference. As a result, liver cells respond to lower blood glucose levels whereas muscles do not. Another distinction is the lack of the glucose 6-phosphatase enzyme in muscle cells, which converts 6-glucose phosphate to inorganic phosphate, which can flow outside the cells in the opposite direction as 6-glucose phosphate. In other words, glucose generated by liver cells can enter the bloodstream, whereas glucose produced by muscles cannot. Muscle cells, on the other hand, can indirectly contribute to the regulation of glucose in the blood, because when muscle cells produce lactate during anaerobic contractions, lactate is transferred to liver cells, which then use gluconeogenesis to synthesize glucose molecules from these lactate molecules. 3 4

**Materials:**

1. 2 Rats of the same age, sex and weight (one fed and one starved for 48 h)
2. 100 ml of 300 g/L KOH
3. Centrifuge tubes
4. Boiling water baths and marbles
5. 100 ml of 95% (v/v) Ethanol
6. 100 ml volumetric flasks
7. 10 ml test tubes
8. 50 ml of 1.2 M HCI
9. 20 ml of Phenol red indicator solution
10. 50 ml of 0.5 M NaOH
11. Glucose oxidase kit

**Methods:**

**I - Isolation of glycogen:**

1. A rat was sacrificed and 1.5 g of liver or skeletal muscle was accurately weighed.

2. Tissue was placed in a calibrated tube (the wall of the tube was marked 10 ml) and the centrifuge tubes were previously weighed with 2 ml KOH and heated in a boiling water bath for 20 min using occasional shaking.

3. The tubes have been cooled in ice for 1 minute.

4. Glycogen was precipitated by adding 4.5 ml of ethanol (95% v/v), stood on ice for 5 minutes, and removed the precipitate by centrifuged (2000 rpm for 5 minutes).

5. The supernatant was discarded, the walls of the centrifuge tubes were dried well and the weight of the pellets was recorded.

6. The weights of glycogen pellet were compared in feed and starved mice.

7. The precipitated glycogen was dissolved in about 5 ml of water with gentle warming, then it was diluted with distilled water to the 10 ml mark and mixed well. In the case of fed animals, the liver sample was quantitatively transferred to a 100 mL volumetric flask and replaced with water.

**II- Hydrolysis and estimation of Glycogen:**

 8. The pipette duplicate 1 mL samples of the glycogen solution into calibrated test tubes at 5 mL.

9. For each sample 1 ml of HCI was added, then a place of marble was added over each tube, and heated in a boiling water bath for 20 min.

10. After hydrolysis, one drop of phenol red indicator was added and carefully neutralized with NaOH until the indicator changed from pink to orange to yellow.

11. It was diluted to 5 ml with distilled water and the glucose content was determined by a glucose oxidase kit.

12. Reagents and samples were brought to room temperature.

13. Pipette into labelled tubes:

**Table\_1:** The amount of cuvettes

|  |  |  |  |
| --- | --- | --- | --- |
| tubes | blank | sample | cal.standard |
| r1.monoreagent | 1.0 ml | 1.0 mL | 1.0 mL |
| sample | - | 10 µL | - |
| cal.standard | - | - | 10 µL |

14. The tubes were mixed and left to stand 10 minutes at room temperature or 5 minutes at 37°C.

15. Absorbance (A) was read for samples and standard at 500 nm against the blank reagent.

* **Results:**

**Table\_2:** Glycogen samples were extracted from different fed and starved rats' tissues and their absorbance values were measured at 500 nm and the data from fed and starved of liver and muscle tissue.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Tissue | Tissue wt.(g) | Glycogen wt. (g) | $$^{Glycogen}/\_{Tissue} \%$$ | Astd | Asample1 | Asample2 |
| Fed liver | **1.5 g** | **0.8 g** | **53.3 %** | **0.314** | **0.094** | **0.097** |
| Starved liver | **1.5 g** | **0.4 g** | **27%** | **0.325** | **0.014** | **0.010** |
| Fed muscles | **1.5 g** | **0.1 g** | **7%** | **0.222** | **0.013** | **0.014** |
| Starved muscles | **1.5 g** | **0.2 g** | **13%** | **0.312** | **0.020** | **0.007** |

**Table\_3:** The concentration of glucose (mg/dL) and the gram of glycogen per 100 g of tissue (mmol/L)

|  |  |  |
| --- | --- | --- |
| Tissue | Concentration of glucose (mg/dL) | grams of glycogen per 100 g of tissue (mmol/L) |
| Fed liver | **30.414** | **1.6880** |
| Starved liver | **3.700** | **0.2049** |
| Fed muscles | **6.081** | **0.3375** |
| Starved muscles | **4.320**  | **0.2401** |

**Discussion:**

Animals have used methods to store energy sources, such as glycogen. Found primarily in the liver and a small proportion in muscle cells, glycogen is broken down into its glucose monomers when necessary to address the body's metabolic demands, a process known as glycogenolysis.

And we used two rat, one fed and the other starved, to see the percentage of glycogen and glucose they have, the no eating will affect the rat? And the proportion of glucose and glycogen we have?

Well, and as we talked previously and as shown in table\_3 the ratio of grams of glycogen per 100 g of tissue (mmol/L), it also shows us that the fed rat has the highest percentage and this is due to the fact that the liver contains a high concentration of glucose and glycogen so that it is 1.6880 mmol/L on the In contrast to a starved rat with 0.2049 mmol/L, there is a very distinct difference between the two because the main enzyme responsible for glycogenolysis is glycogen phosphorylase, which is activated by signal transduction pathways triggered by epinephrine and glucagon, which are secreted into the blood in the case of starvation. On the other hand, these signals inhibit the activity of Glycogen Synthase, which is the main enzyme responsible for the condensation of glucose monomers forming glycogen granules.6

Glycogen breakdown in muscle cells provides an immediate source of glucose-6-phosphate for glycolysis, which provides energy for muscular contraction. So, as shown in table\_3, the percentage of Fed muscles is higher than the percentage of starved muscles, the two ratios should be equal, but the difference between them is 0.0974, although the percentage of glucose in both cases should be equal, but it is okay that the rat are not equal in muscle mass or from It is possible that a rat made an exercise effort.

There is a difference in the ratios between the liver and the muscles, so that the percentage of glucose is largely present in the liver, unlike the muscles, and the reason is that the main organ for storing glycogen is the liver, and that we noticed from the results that we have. The expected results were correct, so that the starved rat has a low glycogen level, while the feeding rate is high, but the muscles should have been equal, but we justified the reason above**.**

**Conclusion:**

To summarize, Glycogen is an energy storage system in animals that is made up of glucose (the most abundant source of energy in all organisms) and is found in the liver and muscles. During starvation, the body consumes Glycogen exclusively from the liver, not from the muscles, which is consumed in the presence of exercise.

**References:**

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**Appendix:**

**Note:** Concentration of glucose standard= 100 mg/dL (5.55 mmol/L)

**Calculation of Fed Muscles:**

* The average of two samples: (0.013+0.014)/2= 0.0135
* $\frac{A sample}{A standard}$ x C standard =

= (0.0135/0.222) \*100 mg/dL

 = 6.0811 mg/dL

🡪 To Calculate the grams of glycogen per 100 g of tissue in glycogen with mole:

= [glucose] \* 0.0555

= 0.3375 mmol/L