**Biology and Biochemistry Department**

**BIOC 312**

**Biochemistry II lab**

**Experiment #6**

**Sec 1**

**Student Name: Meran Nasser**

**Student ID: 1190803**

**Instructor: Dr. Johnny Stiban**

**Teacher Assistance: Mr. Yousef Nammari**

**Partners:**

**Ali Milhem**

**Abdalrahman**

**AbdelFattah**

**Melak Ottallah**

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**Title**

**Metabolic Regulation I – The effects of hormones on rat liver glycogen levels**

**Objective:**

The objective of this experiment is to determine the effects of various hormones, or the lack thereof, on glycogen levels in the liver and skeletal muscles of rats.

**Introduction:**

The autonomic and constantly regulating of glucose in the body occurs at all times of the day. It is important for us to understand how our body uses food to obtain energy naturally, because the dysregulation of blood sugar leads to many different diseases, such as diabetes, for example.3

So Glucose is used as an instant energy source by cells all across the body. A constant glucose content of 60 to 100 mg/dL in blood plasma is required to keep the body working normally.Because muscles use glucose for energy, the body requires a greater concentration during activity or stress. Because the glucose is the preferred fuel for the body since it provides both energy and water via the aerobic metabolism and Krebs cycle.3

It is therefore important to maintain normal blood sugar levels through hormonal control too. When glycogenesis, glycogen is synthesized from glucose in living organisms that are well nourished, resulting in high glucose levels, whereas glycogenolysis maintains glucose levels in the blood when glucose levels are low in starving circumstances (the breakdown of glycogen into glucose). Then there's gluconeogenesis, which involves the production of glucose from non-carbohydrate precursors.1

So when blood glucose levels drop, the liver (the central reserve of glycogen in the body and glycogen is mainly stored in it) after two hours by releasing glycogen (stored glucose) replenishes circulating blood glucose.3

We have the main hormones through which glucose regulation is controlled: adrenaline, insulin and cortisol.

In the beginning we have adrenaline (*epinephrine*), which is secreted from the adrenal medulla in response to states of excitement, tension and emergency situations. It acts as a transmitter in the autonomic nervous system and the brain. Glycogen phosphorylase activity is known to be increased by adrenaline through phosphorylase kinase activation by cAMP. And the adrenaline increases act glycogenolysis and inhibits glycogenesis.2

And secondly, we have Insulin is a peptide hormone with 51 amino acids distributed over two peptide chains and he is anabolic hormone that induces metabolic actions throughout the body. Beta cells are found in the pancreas' exocrine tissue, known as the islets of Langerhans. Insulin is produced by beta cells, which are found in the pancreas. Beta cells regulate insulin production by monitoring the amounts of glucose, amino acids, fatty acids and keto acids in the bloodstream. Insulin's main function is to regulate energy conservation and utilization throughout eating and fasting states. As a result, Insulin's ability to increase glycogen production in muscle and liver is one of its most important impacts on intracellular metabolism. This is accomplished by inducing a net decrease in the level of phosphorylation of glycogen synthase, the rate-limiting enzyme in the glycogen production pathway, resulting in an increase in its activity.4 1

Finally, we have Cortisol so the cortisol inhibits the peripheral utilization of glucose, counteracts insulin, and contributes to hyperglycemia by increasing gluconeogenesis. Cortisol also stimulates glycogen synthesis (glycogenesis) in the liver, allowing glucose to be stored in a form that is easily accessible.5

**Materials:**

1. 7 Rats the same age, sex and weight:

|  |  |  |
| --- | --- | --- |
| RAt | Diet | Treatment |
| 1 | Normal diet | None  |
| 2 | Normal diet | Adrenaline  |
| 3 | Normal diet  | Streptozotocin  |
| 4 | 6 hours starvation  | None |
| 5 | 48 hours starvation  | None  |
| 6 | 72 hours starvation  | None |
| 7 | 48 hours starvation  | Cortisol  |

1. *Adrenaline treatment:* subcutaneous injection of 10 µg/100 g weight of rat. The rat must be sacrificed after two minutes of the injection.
2. *Cortisol treatment:* subcutaneous injection, every two hours for 6 hours, 1.2 mg/100 g weight of rat.
3. *Streptozotocin treatment:* prepare STZ in 0.1 M citrate buffer, pH 4.5. Daily intraperitoneal injection of 5 mg/100 g weight of rat for 5 days.
4. 100 ml of 10% TCA
5. 100 ml of 5% TCA
6. Centrifuge tubes
7. 100 ml of 95% (v/v) Ethanol

**Methods:**

1. Rat were sacrificed and weighed 1.5 g of liver and 1.5 g of skeletal muscle.

2. It was cut into small pieces. Homogenized with 1.5 ml of cold 10% TCA (to precipitate most of the liver proteins).

3. Centrifuge homogenization at 3000 rpm for 5 min at 4 °C. The supernatant was poured into a 50 mL Falcon tube.

4. The homogenization tube was rinsed 1.5 ml of 5% TCA. This rinsing fluid was added to centrifuge tubes containing residues from the first centrifugation. Stir the residue and re-centrifuge at 3000 rpm for 5 min. The supernatant was collected in the previously collected tube. The granules were discarded.

5. The total volume (3 ml) has been recorded. So three times this volume (9 mL) of cold 95% ethanol was added, slowly, with stirring, to the supernatant. The glycogen was allowed to precipitate for 5 minutes by placing it on ice. If not, and a little NaCl was added, warm the tube in a water bath at 37°C.

6. In pre-weighed centrifuge tubes, the suspension was rotated at 3000 rpm for 3 min to collect glycogen granules.

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

**Results:**

**Table\_1:** The conditions in which the rat lived was and the percentage of glycogen weight (mg), in addition to the yield of glycogen in the liver (mg/g).

|  |  |  |
| --- | --- | --- |
| **# RAT** | **Glycogen weight (mg)** | **Glycogen / liver (mg/g)** |
| **1 Normal**  | 0.4  | 266.6 mg/g |
| **2 Adrenaline**  | 0.2  | 133.3 mg/g |
| **3 STZ** | 0.5  | 333.3 mg/g |
| **4 6hr** | 0.1  | 66.70 mg/g |
| **5 48hr** | 0.3  | 200.0 mg/g |
| **6 74hr** | 0.1  | 66.70 mg/g |
| **7 48hr+cortisol** | 0.3  | 200.0 mg/g |

**Discussion:**

In this experiment, we starved rat and gave 3 different hormones in order to control glucose regulations so that they are maintained by hormonal control of blood sugar levels.

At first, we brought the normal rat as it appears in Table\_1 and measured the weight of glycogen so that we have 0.4 g, which is a possible percentage and not bad.

As it appears to us in the second rat, he was given adrenaline to so the percentage of glycogen is decreased to 0.2 g and this is a logical result as well. We gave him the adrenaline and dissected him within two minutes, as his heart was also very fast, and the reason was because the adrenalin was working to move a glycogen from to sources from stores in livers.

\* All Rats had the same conditions, same fats, age, and so on.

Next, we have a rat that was injected with streptozotocin (diabetes models), a hormone that kills β cells and thus leads to an inability to manufacture insulin because streptozotocin cannot manufacture it and thus inability to regulate blood sugar and thus glycogen Synthase is lower in the absence of insulin.

And we had a glycogen weight equal to 0.5 g, and this is an illogical number. The reason is because insulin performs a storage of glycogen, and when the insulin lower so the glycogen is lower. and therefore the value of the glycogen weight for the rat with the streptozotocin hormone should be less than the value of the glycogen weight of the normal rat.

And then we have 3 rats that were starved at different hours from each other, as shown in Table\_1 the value of the glycogen weight for the first rat was 0.1 g and the second was 0.3 g. As we can see, it is clear that we have an error rate here. This is because the more hours of fasting, the less glycogen is supposed to be, but it is possible that the reason for this error is that the rat here were opposite, i.e. the rat was dissected for 24 hours, and it was already the rat that was staved for 48 hours, and so on. As for the value of the sixth rat, which is the third starved rat (72 hours), its result was expected and logical as well.

Finally, we will talk about the seventh rat, which is the rat that has been starved for 48 hours and has the hormone cortisol, which increases the proportion of glycogen in the liver. Which causes a higher percentage of glycogen weight starved 48 hours + cortisol than the proportion of the starved rat only 48 hr. Therefore, the result is logical there as well, because the mechanism of action of cortisol is the opposite of insulin. This is because insulin reduces glycogen when he reduced, while cortisol increases glycogen when he increased.

One of the errors expected in the experiment is that the tube of homogenize was not 100% clean, which caused a percentage of the error, and it is also possible that the tube had a little fluid left in it, which thus increased my percentage of glycogen. It is possible that the covers of the falcon tubes have been replaced to each other and therefore the error rate as well. And there are random errors from the measurement precision balance, it is possible that the error was caused by it. One of the possible errors is that the micropipette was not accurate or had a specific malfunction.

\* Ethanol has been used to precipitate glycogen faster and better and to break down our liver.

**Conclusion:**

In conclusion, by giving rat different hormones, we can learn how glycogen levels change in the liver so that some rats were containing these hormones or were starved for hours different from each other, which led to a good understanding of how these hormones affect the levels of glycogen in the liver.

**References:**

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**Appendix:**

In Table\_1:

Glycogen/live (mg/g)

For example, the glycogen weight = 0.4 | liver weight = 1.5 g So

= $\frac{0.4\*1000}{1.5}=266.6 mg/g$