# Biology 347 General Physiology Lab
# Hormonal Control of Blood Glucose Levels

## Objectives

- Students will appreciate the importance, as well as the sensitivity, of research and teaching utilizing vertebrate animals
- Students will gain exposure on animal handling including:
    - weighing and anesthetizing awake animals
    - verifying that anesthesia is sufficient to prevent pain
    - calculating appropriate doses of anesthesia and hormones
    - appreciating the importance of careful data collection
- Students will gain an understanding of the hormonal control of blood glucose level in the vertebrate

## Preliminary Information about Laboratory Exercise

All animal handling, in research, agriculture, etc. must be done humanely. At Saint Louis University, and everywhere, no research or teaching project may be undertaken without specific approval. The Department of Comparative Medicine, John Long, DVM, Chair, oversees animal work at SLU, and they answer directly to Fr. Biondi, President of SLU. A proposal for each project must address background, references, methods, significance, whether the work has been done before (for research, requiring a literature search) or whether computer animations can substitute (for teaching), how to minimize the number of animals used, whether the animals can be used in several projects (animal conservation), qualifications of personnel handling animals, and, perhaps first and foremost, how to ensure that there is minimal pain or distress. All personnel must be trained and refreshed. Each proposal is evaluated rigorously by a diverse committee including faculty from Medical and Frost campuses as well as non-academic and non-SLU people. All projects must be renewed annually with a statement assuring the committee that there were no problems. All projects must be applied for again after 3 years. The committee site-visits all labs and all animal quarters regularly. The department solicits any concerns from anybody. All university activities are site-visited and evaluated by the appropriate national agencies. Learn more by visiting the Comparative Medicine site. Your laboratory is project #1622 "Undergraduate Physiology Laboratory on Blood Glucose," approved 12/27/07.

## Introduction

Hormones are chemical messengers that are secreted by ductless endocrine glands or tissues, directly into the circulatory system. Hormones are responsible for the homeostatic control of such processes as growth, maturation, reproduction and metabolism.

Available carbohydrate energy is circulated in the plasma as glucose. The utilization of glucose by body tissues can be gauged by the circulating levels of glucose in the plasma. Low levels of glucose in the plasma may indicate fasting or high utilization of glucose by the tissues, while high plasma glucose levels follow a meal or may indicate a decreased utilization by the bulk of the body tissues. This exercise is designed to demonstrate the importance of two hormones, insulin and glucagon, in the regulation of plasma glucose levels.

Insulin is a protein hormone that is produced and released by the beta cells of the pancreatic islets. Type I (juvenile onset) diabetes mellitus is thought to be an autoimmune disease in which the beta cells are destroyed, leading to low insulin and high blood sugar; replacement must be by injection since insulin is a protein. Diabetes is such an important health problem that it is mentioned in half of the chapters of your test (Silverthorn). High blood sugar, hyperglycemia, leads to glucose in the urine by swamping the mechanism of glucose recovery in the kidney. Later this semester, the kidney lab will test for glucose in the urine. Once in the plasma, insulin increases the uptake and utilization of glucose by insulin-sensitive tissues (muscle, fat and liver). In extreme cases, when insulin causes glucose to be moved out of the plasma and into these "target tissues," it results in a condition known as hypoglycemia (low blood glucose). Too much insulin can lead to such extreme hypoglycemia that it is called insulin shock caused by low glucose availability in the brain (combined with the brain's glucose uptake not being insulin dependent plus the brain's heavy reliance on glucose). Insulin is typically released following feeding and, at that time, large amounts of glucose are available for storage and utilization by the bulk of the body tissues.

Glucagon is released by the alpha cells of the pancreatic islets when plasma glucose levels are low. Once in the plasma, glucagon acts primarily on the liver to mobilize glucose from the glycogen stores and release glucose from muscle, fat and

hepatic tissues. In extreme cases, the resulting release causes a large increase in the plasma levels of glucose or the condition hyperglycemia. Glucagon is released during periods of fasting in response to low plasma glucose levels to mobilize glucose needed to maintain neural and other vital tissues.

## Procedure: Anesthetization of Animals

Prior to surgery, the subject must be anesthetized to minimize any pain or discomfort. In this exercise we will use ketamine HCl (86.98 mg/kg) plus xylazine HCl (13.04 mg/kg). The dose of 0.10 ml/20 grams body weight will be administered intraperitoneally with a 25 gauge needle.

Determine the weight of your animal in g: _____

Use the above information to determine the amount of ketamine and acepromazine cocktail needed to anesthetize the animal.

---

1. To administer the injection, gently lift the mouse by the tail and place it on a cage lid. This gives the mouse something to grip and lulls it into a sense of security. Grip the loose skin of the neck between the thumb and first finger to immobilize the head. With the head immobilized, extend the tail to draw the skin taut over the abdomen. This may be done with one hand by gripping the tail with your little finger. If your hands are too small to fully extend the mouse or if you would prefer to use a two-hand grip, have your lab partner administer the injection.

2. Get the right amount into the syringe without air. Insert the hypodermic needle into the lower left quadrant of the abdomen. A slight back pressure should reveal no blood. Inject the appropriate quantity of drug. The needle should be held almost perpendicular to the skin, approximately halfway between the pelvis and sternum and slightly to the side of the midline, in order to avoid puncturing the bladder.

3. After administering the anesthetic, place the mouse into a cage and wait until the drug has taken effect. Be patient, it takes time for the drug to be absorbed into the systemic circulation. If the mouse does not go under after the initial dose it may be necessary to give a supplemental injection.

***** Drs. Stark and Shornick will demonstrate the procedure for injecting the mice with the necessary amount of anesthetic for all groups. *****

4. When there are no signs of voluntary movement, remove the mouse from the cage and place it on a lab towel. It is extremely important to verify that the animal does not suffer pain. Test for reflexive movement by foot pinch. If no movement is observed then the subject is ready for the procedure. Do not rush the procedure.

5. Immediately snip a mm or 2 off the tip of the mouse's tail. If necessary, milk a drop of blood from the cut end onto the reagent strip for the glucose meter. Get a reading and compare yours with those from other lab groups. This is your control reading, and it is very important. If you have any doubt, do it again.

6. Record your control readings in Table 1.

## Procedure: Injection of Insulin

1. Inject 0.2-0.3 U of insulin in 0.2ml of physiological saline solution I.P. Note the time of injection. Begin timing for 30 minutes.

2. Test another drop of blood from the mouse's tail. (Usually, the scab can be pinched off so that another snip is not necessary). Record your data in Table 1.

   Time of Injection: _____

**Procedure: Injection of Glucagon**

1. After the insulin reading, administer 0.2 ml of glucagon I.P. (0.05 mg/0.2 ml physiological saline). Record the time of injection. Wait an additional 30 minutes.

2. Test another drop for the glucagon experimental sample. Record your data in Table 1.

   Time of Injection: _____

3. At the completion of the exercise the mouse is to be returned to its cage for recovery.

4. Compile data from all groups and Record in Table 2.

**Table 1: Hormonal Control of Blood Glucose Levels (mg/dL)**

| | **Blood Glucose Levels (mg/dL)** |
|---|---|
| **Control (initial reading)** | |
| **30 minutes post Insulin Injection** | |
| **30 minutes post Glucagon Injection** | |

**Table 2: Hormonal Control of Blood Glucose Levels (mg/dL)- Group Data**

| | **Blood Glucose Levels (mg/dL)** | | |
|---|---|---|---|
| | **Control** | **30 min post Insulin Injection** | **30 min post Glucagon Injection** |
| Group 1 | | | |
| Group 2 | | | |
| Group 3 | | | |
| Group 4 | | | |
| Group 5 | | | |
| Group 6 | | | |