

Endocrine System Physiology



The endocrine system regulates the functioning of every cell, tissue, and organ in the body. It acts to maintain a stable internal body environment, regardless of changes occurring within or outside of the body. Endocrine cells have the ability to sense and respond to changes via the excretion of specific chemicals known as **hormones**. Hormones are carried in the blood, usually attached to specific plasma proteins, and circulate around the body. When the hormone-protein complex reaches a **target cell** (the cell at which a chemical message is aimed), the hormone detaches from the protein and enters the cell to induce a specific reaction.

Hormones work in different ways, depending upon their chemical structures. For example, *polypeptide hormones*, composed of chains of amino acids, work by first attaching to a protein receptor in the cell membrane, initiating a series of reactions in the membrane resulting in *cyclic adenosine monophosphate* (cAMP) entering the cell. The entrance of this chemical into the cell induces the cell to work harder and faster. *Steroid hormones* and *thyroxine* (a hormone secreted by the thyroid, which we will be examining in detail shortly) enter the cell to attach to a cytoplasmic receptor. The hormone-receptor complex then enters the nucleus of the cell to attach to specific points on the DNA. Each attachment causes the production of a specific mRNA, which then moves to the cytoplasm to be translated into a specific protein.

Most regulation of hormone levels in the body is conducted by **negative feedback**: if a particular hormone is needed, production of that hormone will be stimulated; if there is enough of a particular hormone present, production of that hormone will be inhibited. In a few very specific instances, hormonal output is controlled by *positive feedback* mechanisms. One such instance is the output of the posterior pituitary hormone *oxytocin*. This hormone causes the muscle layer of the uterus, the *myometrium*, to contract during childbirth. Contraction of the myometrium causes additional oxytocin to be released to aid in the contraction, regardless of the amount of hormone already present.

Studying the effects of hormones on the body is difficult to do in a wet lab, since experiments can often take days, weeks, or even months to complete, and are quite expensive. In addition, live animals may need to be sacrificed, and technically difficult surgical procedures are sometimes necessary. The PhysioEx simulations you will be using in this lab will allow you to study the effects of given hormones on the body by using “virtual” animals rather than live ones. You will be able to carry out delicate surgical techniques with the click of a button. You will also be able to complete experiments in a fraction of the time that it would take in an actual wet lab environment.

Objectives

1. To define the following: hormones, target cell, negative feedback, metabolism, thyroxine, thyroid stimulating hormone (TSH), thyrotropin releasing hormone (TRH), hypothalamus, hypothalamic pituitary portal system, portal vein, hormone replacement therapy, diabetes type I, diabetes type II, glucose standard curve
2. To give examples of how negative feedback loops regulate hormone release
3. To explain the role of thyroxine in maintaining an animal's metabolic rate
4. To explain the effects of thyroid stimulating hormone (TSH) on an animal's metabolic rate
5. To understand the role of the hypothalamus in the regulation of thyroxine and TSH production
6. To understand how hypothalamic hormones reach the pituitary gland
7. To understand how estrogen affects uterine tissue growth
8. To explain how hormone replacement therapy works
9. To explain why insulin is important and how it can be used to treat diabetes

Hormones and Metabolism

Metabolism is the broad term used for all biochemical reactions occurring in the body. Metabolism involves *catabolism*, a process by which complex materials are broken down into simpler substances, usually with the aid of enzymes found in body cells. Metabolism also involves *anabolism*, in which the smaller materials are built up by enzymes to build larger, more complex molecules. When bonds are broken in catabolism, energy that was stored in the bonds is released for use by the cell. When larger molecules are made, energy is stored in the various bonds formed. Some of the energy liberated may go to the formation of ATP, the energy-rich material used by the body to run itself. However, not all of the energy liberated goes into this pathway. Some of that energy is given off as body heat. Humans are *homeothermic* animals, meaning they have a fixed body temperature. Maintaining this temperature is very important to maintaining the metabolic pathways found in the body.

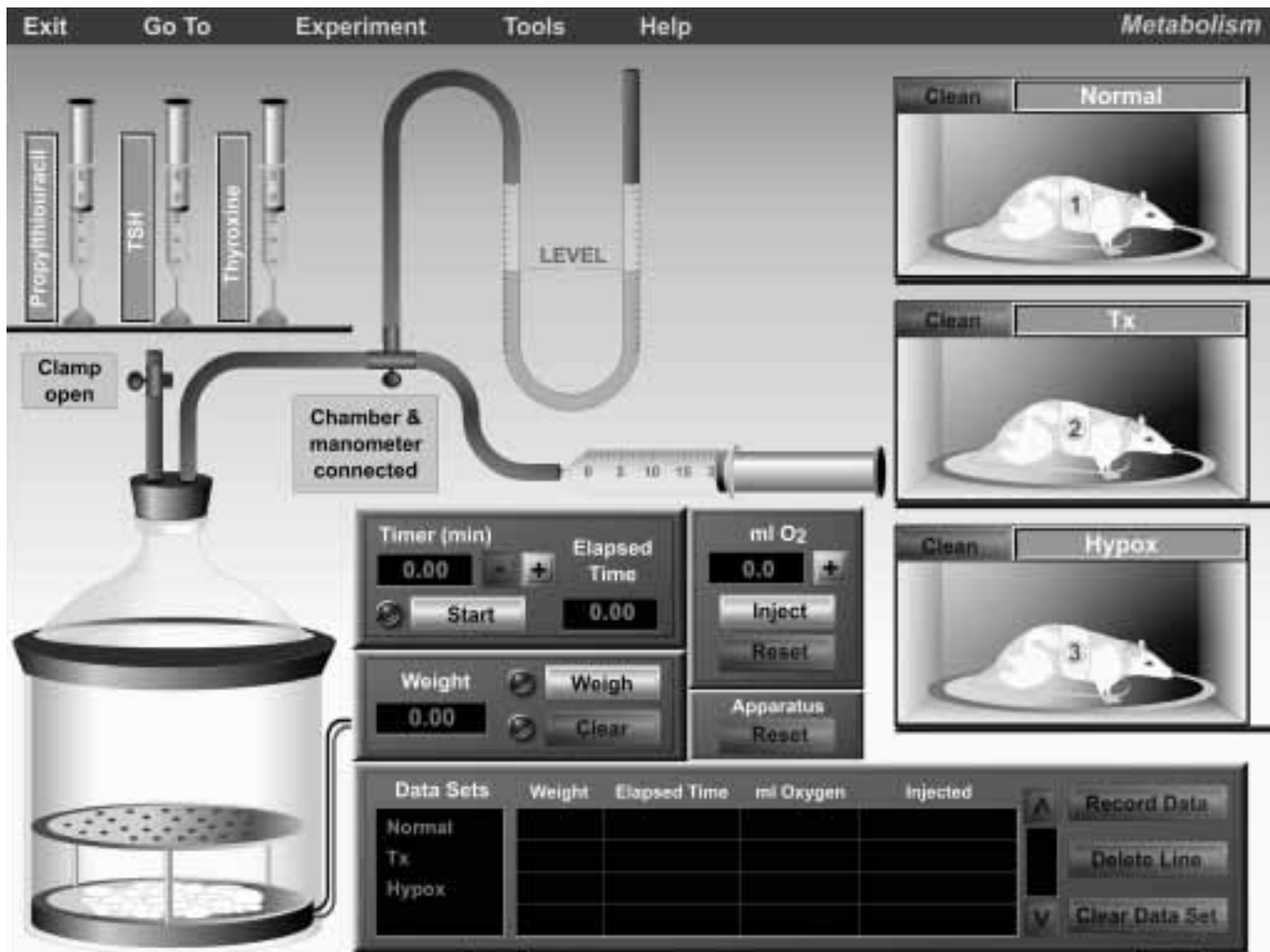


Figure 4.1 Opening screen of the Metabolism experiment.

The most important hormone in maintaining metabolism and body heat is **thyroxine**. Also known as *tetraiodothyronine*, or T_4 , thyroxine is secreted by the thyroid gland, located in the neck. However, production of thyroxine is really controlled by the pituitary gland, which secretes **thyroid stimulating hormone (TSH)**. TSH is carried by the blood to the thyroid gland (its *target tissue*) and causes the thyroid to produce more thyroxine.

It is also important to understand the role of the **hypothalamus** in thyroxine and TSH production. The hypothalamus, located in the brain, is a primary endocrine gland that secretes several hormones affecting the pituitary gland (also located in the brain.) Among these hormones is **thyrotropin releasing hormone (TRH)**, which stimulates production of TSH in the pituitary gland. If the hypothalamus determines that there is not enough thyroxine circulating to maintain the body's metabolism, it will secrete TRH to stimulate production of TSH by the pituitary gland, which in turn will stimulate production of thyroxine by the thyroid (a classic example of a negative feedback loop). TRH travels from the hypothalamus to the pituitary gland via the **hypothalamic-pituitary portal system**, a specialized arrangement of blood vessels

consisting of a single **portal vein** that connects two capillary beds. The hypothalamic-pituitary portal system transports many other hormones from the hypothalamus to the pituitary gland. Primarily, the hormones secreted by the hypothalamus are *tropic* (or *trophic*) hormones, which are hormones that stimulate or inhibit the secretion of other hormones. TRH is an example of a tropic hormone, since it stimulates the release of TSH (which is itself a tropic hormone, since it stimulates the release of thyroxine).

In the following experiments you will be investigating the effects of thyroxine and TSH on an animal's metabolic rate. To begin, follow the instructions for starting PhysioEx in the "Getting Started" section at the front of this manual. From the main menu, select **Endocrine System Physiology**. The opening screen will appear in a few seconds (see Figure 4.1). Select **Balloons On** from the Help menu for help identifying the equipment onscreen (you will see labels appear as you roll over each piece of equipment). Select **Balloons Off** to turn this feature off before you begin the experiments.

Study the screen. You will see a jar-shaped chamber to the left, connected to a *respirometer-manometer apparatus* (consisting of a U-shaped tube, a syringe, and associated tub-

ing). You will be placing animals—in this case, rats—in the chamber in order to gather information about how thyroxine and TSH affect their metabolic rates. Note that the chamber also includes a weight scale, and that next to the chamber is a timer for setting and timing the length of a given experiment. Under the timer is a weight display.

Two tubes are connected to the top of the chamber. The left tube has a clamp on it that can be opened or closed. Leaving the clamp open will allow outside air into the chamber; closing the clamp will create a closed, airtight system. The other tube leads to a **T-connector**. One branch of the T leads to a fluid-containing U-shaped tube, called a *manometer*. As an animal uses up the air in the closed system, this fluid will rise in the left side of the U-shaped tube and fall in the right.

The other branch of the T-connector leads to a syringe filled with air. Using the syringe to inject air into the tube, you will measure the amount of air that is needed to return the fluid columns to their original levels. This measurement will be equal to the amount of oxygen used by the animal during the elapsed time of the experiment. Soda lime, found at the bottom of the chamber, absorbs the carbon dioxide given off by the animal so that the amount of oxygen used can be measured easily. The amount of oxygen used by the animal, along with its weight, will be used to calculate the animal's metabolic rate.

Also on the screen are three white rats in their individual cages. These are the specimens you will use in the following experiments. One rat is **normal**; the second is **thyroidectomized** (abbreviated on the screen as **Tx**)—meaning its thyroid has been removed; and the third is **hypophysectomized** (abbreviated on the screen as **Hypox**)—meaning its pituitary gland has been removed. The pituitary gland is also known as the *hypophysis*, and removal of this organ is called a *hypophysectomy*.

To the top left of the screen are three syringes containing various chemicals: propylthiouracil, thyroid stimulating hormone (TSH), and thyroxine. TSH and thyroxine have been previously mentioned; propylthiouracil is a drug that inhibits the production of thyroxine by blocking the incorporation of iodine into the hormone. You will be performing four experiments on each animal: 1) you will determine its baseline metabolic rate, 2) you will determine its metabolic rate after it has been injected with thyroxine, 3) you will determine its metabolic rate after it has been injected with TSH, and 4) you will determine its metabolic rate after it has been injected with propylthiouracil.

You will be recording all of your data on **Chart 1** (see p. 34). You may also record your data onscreen by using the equipment in the lower part of the screen, called the *data collection unit*. This equipment records and displays the data you accumulate during the experiments. The data set for **Normal** should be highlighted in the **Data Sets** window, since you will be experimenting with the normal rat first. The **Record Data** button lets you record data after an experimental trial. Clicking the **Delete Line** or **Clear Data Set** buttons erases any data you want to delete.

Activity 1: Determining the Baseline Metabolic Rates

First, you will determine the baseline metabolic rate for each rat.

- Using the mouse, click and drag the **normal** rat into the chamber and place it on top of the scale. When the animal is in the chamber, release the mouse button.
- Be sure the clamp on the left tube (on top of the chamber) is open, allowing air to enter the chamber. If the clamp is closed, click on it to open it.
- Be sure the indicator next to the T-connector reads “Chamber and manometer connected.” If not, click on the **T-connector knob**.
- Click on the **Weigh** button in the box to the right of the chamber to weigh the rat. Record this weight in the **Baseline** section of **Chart 1** for “Weight.”
- Click the **(+)** button on the Timer so that the Timer display reads 1.00.
- Click on the clamp to close it. This will prevent any outside air from entering the chamber, and ensure that the only oxygen the rat is breathing is the oxygen inside the closed system.
- Click **Start** on the Timer display. You will see the elapsed time appear in the “Elapsed Time” display. Watch what happens to the water levels in the U-shaped tube.
- At the end of the 1-minute period, the timer will automatically stop. When it stops, click on the **T-connector knob** so that the indicator reads “Manometer and syringe connected.”
- Click the clamp to open it so that the rat can once again breathe outside air.
- Click the **(+)** button under “ml O₂” below the syringe, so that the display reads 1.0 ml. Then click **Inject**, and watch what happens to the fluid levels. Continue clicking the **(+)** button and injecting air until the fluid in the two arms of the U-tube is level again. How many ml of air needed to be added to level the fluid in the two arms? (This is equivalent to the amount of oxygen that the rat used up during the 1 minute in the closed chamber.) Record this measurement in the **Baseline** section of **Chart 1** for “ml O₂ used in 1 minute.”
- Determine the oxygen consumption per hour for the rat. Use the following formula:

$$\frac{\text{ml O}_2 \text{ consumed}}{1 \text{ minute}} \times \frac{60 \text{ minutes}}{\text{hr}} = \text{ml O}_2/\text{hr}$$

Record this data in the Baseline section of Chart 1 for “ml O₂ used per hour.”

- Now that you have the amount of oxygen used per hour, determine the metabolic rate per kilogram of body weight by using the following formula (Note that you will need to convert the weight data from g to kg before you can use the formula):

$$\text{Metabolic rate} = \frac{\text{ml O}_2/\text{hr}}{\text{wt. in kg}} = \text{_____ ml O}_2/\text{kg/hr}$$

Record this data in the Baseline section of Chart 1 for “Metabolic rate.”

- Click **Record Data**.
- Click and drag the rat from the chamber back to its cage.
- Click the **Reset** button in the box labeled *Apparatus*.

	Normal Rat	Thyroidectomized Rat	Hypophysectomized Rat
Baseline			
Weight	_____ grams	_____ grams	_____ grams
ml O ₂ used in 1 minute	_____ ml	_____ ml	_____ ml
ml O ₂ used per hour	_____ ml	_____ ml	_____ ml
Metabolic rate	_____ ml O ₂ /Kg./Hr.	_____ ml O ₂ /Kg./Hr.	_____ ml O ₂ /Kg./Hr.
With Thyroxine			
Weight	_____ grams	_____ grams	_____ grams
ml O ₂ used in 1 minute	_____ ml	_____ ml	_____ ml
ml O ₂ used per hour	_____ ml	_____ ml	_____ ml
Metabolic rate	_____ ml O ₂ /Kg./Hr.	_____ ml O ₂ /Kg./Hr.	_____ ml O ₂ /Kg./Hr.
With TSH			
Weight	_____ grams	_____ grams	_____ grams
ml O ₂ used in 1 minute	_____ ml	_____ ml	_____ ml
ml O ₂ used per hour	_____ ml	_____ ml	_____ ml
Metabolic rate	_____ ml O ₂ /Kg./Hr.	_____ ml O ₂ /Kg./Hr.	_____ ml O ₂ /Kg./Hr.
With Propylthiouracil			
Weight	_____ grams	_____ grams	_____ grams
ml O ₂ used in 1 minute	_____ ml	_____ ml	_____ ml
ml O ₂ used per hour	_____ ml	_____ ml	_____ ml
Metabolic rate	_____ ml O ₂ /Kg./Hr.	_____ ml O ₂ /Kg./Hr.	_____ ml O ₂ /Kg./Hr.

16. Now repeat steps 1–15 for the thyroidectomized (“Tx”) and hypophysectomized (“Hypox”) rats. Record your data in the **Baseline** section of **Chart 1** under the corresponding column for each rat. Be sure to highlight **Tx** under **Data Sets** (on the data collection box) before beginning the experiment on the thyroidectomized rat; likewise, highlight **Hypox** under **Data Sets** before beginning the experiment on the hypophysectomized rat.

How did the metabolic rates of the three rats differ?

Why did the metabolic rates differ?

If an animal has been thyroidectomized, what hormone(s) would be missing from its blood?

As a result of the missing hormone(s), what would the overall effect on the body be?

How could you treat a thyroidectomized animal so that it functioned like a “normal” animal?

If an animal has been hypophysectomized, what effect would you expect to see in the hormone levels in its body?

What would be the effect of a hypophysectomy on the metabolism of an animal?

Activity 2:
Determining the Effect of Thyroxine on Metabolic Rate

Next you will investigate the effects of **thyroxine** injections on the metabolic rates of all three rats.

Please note that in a wet lab environment you would normally need to inject thyroxine (or any other hormone) into a rat *daily* for at least 1–2 weeks in order for any response to be seen. However, in the following simulations you will only inject the rat once and will be able to witness the same results as if you had administered multiple injections over the course

of several weeks. In addition, by clicking the **Clean** button while a rat is inside its cage, you can immediately remove all residue of any previously injected hormone from the rat and perform a new experiment on the same rat. In a real wet lab environment you would need to either wait weeks for hormonal residue to leave the rat’s system or use a different rat.

1. Select a rat to test. You will eventually test all three, and it doesn’t matter what order you test them in. Under **Data Sets**, highlight **Normal**, **Tx**, or **Hypox** depending on which rat you select.
2. Click the **Reset** button in the box labeled *Apparatus*.
3. Click on the syringe labeled **thyroxine** and drag it over to the rat. Release the mouse button. This will cause thyroxine to be injected into the rat.
4. Click and drag the rat back into the chamber. Perform steps 1–12 of Activity 1 again, except that this time, record your data in the **With Thyroxine** section of Chart 1.
5. Click **Record Data**.
6. Click and drag the rat from the chamber back to its cage, and click **Clean** to cleanse it of all traces of thyroxine.
7. Now repeat steps 1–6 for the remaining rats. Record your data in the **With Thyroxine** section of Chart 1 under the corresponding column for each rat.

What was the effect of thyroxine on the normal rat’s metabolic rate? How does it compare to the normal rat’s baseline metabolic rate?

Why was this effect seen?

What was the effect of thyroxine on the thyroidectomized rat’s metabolic rate? How does it compare to the thyroidectomized rat’s baseline metabolic rate?

Why was this effect seen?

What was the effect of thyroxine on the hypophysectomized rat's metabolic rate? How does it compare to the hypophysectomized rat's baseline metabolic rate?

Why was this effect seen?

Activity 3:
Determining the Effect of TSH on Metabolic Rate

Next you will investigate the effects of TSH injections on the metabolic rates of the three rats. Select a rat to experiment on first, and then proceed.

1. Under **Data Sets**, highlight **Normal**, **Tx**, or **Hypox**, depending on which rat you are using.
2. Click the **Reset** button in the box labeled *Apparatus*.
3. Click and drag the syringe labeled **TSH** over to the rat and release the mouse button, injecting the rat.
4. Click and drag the rat into the chamber. Perform steps 1–12 of Activity 1 again. Record your data in the **With TSH** section of Chart 1.
5. Click **Record Data**.
6. Click and drag the rat from the chamber back to its cage, and click **Clean** to cleanse it of all traces of TSH.

7. Now repeat this activity for the remaining rats. Record your data in the **With TSH** section of Chart 1 under the corresponding column for each rat.

What was the effect of TSH on the normal rat's metabolic rate? How does it compare to the normal rat's baseline metabolic rate?

Why was this effect seen?

What was the effect of TSH on the thyroidectomized rat's metabolic rate? How does it compare to the thyroidectomized rat's baseline metabolic rate?

Why was this effect seen?

What was the effect of TSH on the hypophysectomized rat's metabolic rate? How does it compare to the hypophysectomized rat's baseline metabolic rate?

Why was this effect seen?

Activity 4:
Determining the Effect of Propylthiouracil on Metabolic Rate

Next you will investigate the effects of propylthiouracil injections on the metabolic rates of the three rats. Keep in mind that propylthiouracil is a thyroxine inhibitor.

Select a rat to experiment on first, and then proceed.

1. Under **Data Sets**, highlight **Normal, Tx, or Hypox**, depending on which rat you are using.
2. Click the **Reset** button in the box labeled *Apparatus*.
3. Click and drag the syringe labeled **Propylthiouracil** over to the rat and release the mouse button, injecting the rat.
4. Click and drag the rat into the chamber. Perform steps 1–12 of Activity 1 again, except this time record your data in the **With Propylthiouracil** section of Chart 1.
5. Click **Record Data**.
6. Click and drag the rat from the chamber back to its cage, and click **Clean** to cleanse it of all traces of propylthiouracil.
7. Now repeat this activity for the remaining rats. Record your data in the **With Propylthiouracil** section of Chart 1 under the corresponding column for each rat.
8. Click **Tools** → **Print Data** to print your data.

What was the effect of propylthiouracil on the normal rat’s metabolic rate? How does it compare to the normal rat’s baseline metabolic rate?

Why was this effect seen?

What was the effect of propylthiouracil on the thyroidectomized rat’s metabolic rate? How does it compare to the thyroidectomized rat’s baseline metabolic rate?

Why was this effect seen?

What was the effect of propylthiouracil on the hypophysectomized rat’s metabolic rate? How does it compare to the hypophysectomized rat’s baseline metabolic rate?

Why was this effect seen?

Hormone Replacement Therapy

Ovaries are stimulated by **follicle stimulating hormone (FSH)**, released from the pituitary gland, to get ovarian follicles to develop so that they can ovulate and perhaps be fertilized. While the follicles are developing, the follicular cells that form around the oocyte produce the hormone **estrogen**. One main target tissue for estrogen is the uterus, and the action of estrogen is to enable the uterus to grow and develop so that it may receive fertilized eggs for implantation. *Ovariectomy*, the removal of ovaries, will remove the source of estrogen and cause the uterus to slowly atrophy.

FSH and the subsequent estrogens are also regulated by a negative feedback system. Recall that hormone levels are monitored by the hypothalamus in the brain. If the hypothalamus determines that not enough FSH is present, it will release **gonadotropin releasing hormone (GnRH)** to stimulate FSH production in the pituitary gland. GnRH will be transported from the hypothalamus to the pituitary gland via the hypothalamic-pituitary portal system. Birth control pills

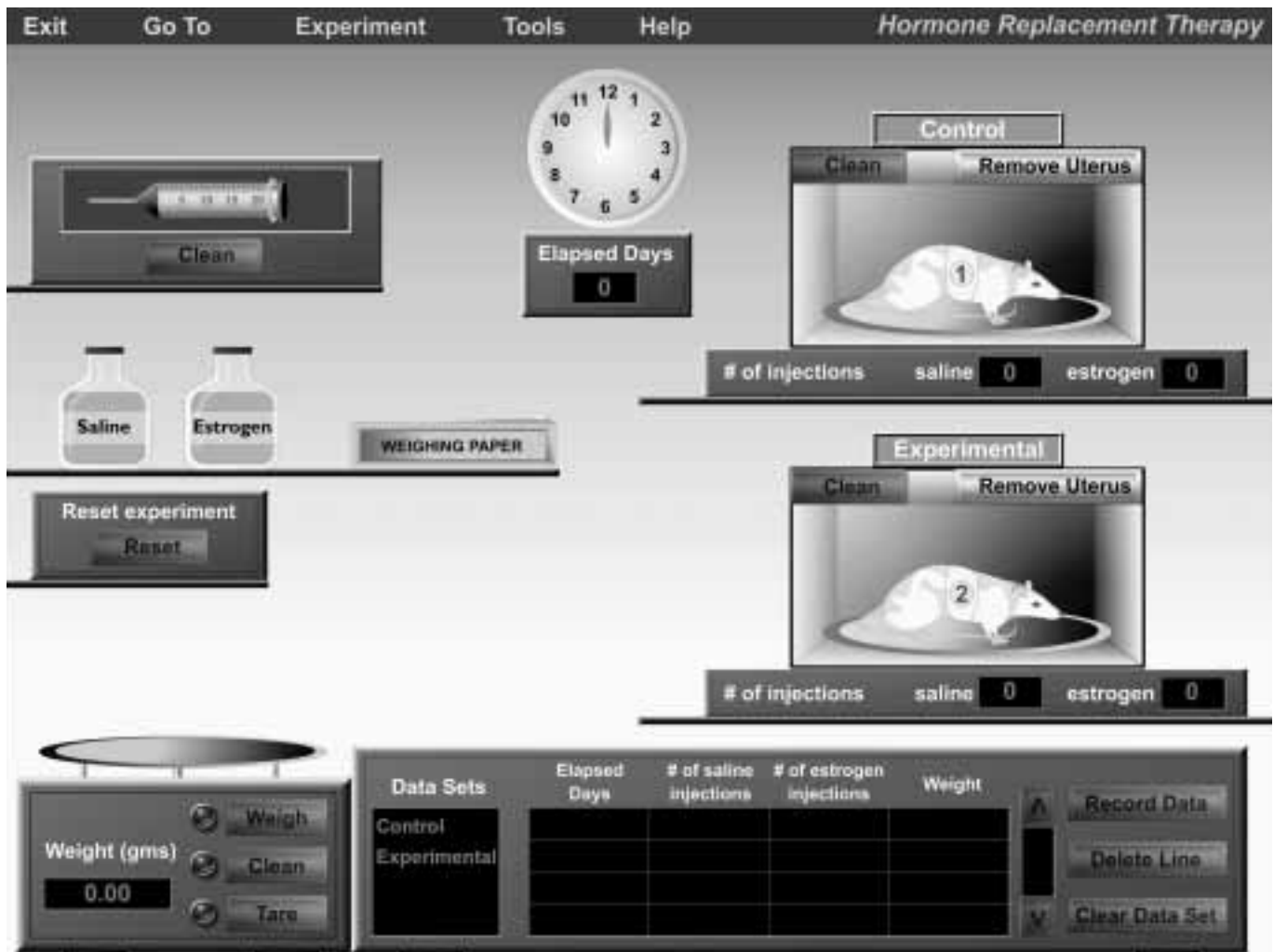


Figure 4.2 Opening screen of the Hormone Replacement Therapy experiment.

work by employing the negative feedback loop. The pills release high levels of estrogen into the body, which block the release of FSH from the pituitary gland so that no new oocyte is developed and ovulated. A similar process occurs during pregnancy: high levels of estrogen produced during pregnancy block the release of additional FSH.

In this activity you will re-create a classic endocrine experiment and examine how **estrogen** affects uterine tissue growth. You will be working with two female rats, both of which have been ovariectomized and, as a result, are no longer producing estrogen. You will administer **hormone replacement therapy** to one rat by giving it daily injections of estrogen. The other rat will serve as your “control” and receive daily injections of saline. You will then remove the uterine tissues from both rats, weigh the tissues, and compare them to determine the effects of hormone replacement therapy.

In the first ovariectomized rat, what do you think will happen to the uterus if estrogen therapy is administered?

In the second ovariectomized rat, what do you think the administration of saline will do to the uterus?

Start by selecting **Hormone Replacement Therapy** from the **Experiment** menu. A new screen will appear (Figure 4.2), showing the two ovariectomized rats in cages. (Please note that if this were a wet lab, the ovariectomies would need to have been performed on the rats a month or more prior to the rest of the experiment in order to ensure that no residual hormones remained in the rats' systems.) Also on screen are a bottle of saline, a bottle of estrogen, a syringe, a box of weighing paper, and a weighing scale.

Proceed carefully with this experiment. Each rat will disappear from the screen once you remove its uterus, and it cannot be brought back unless you restart the experiment. This replicates the situation you would encounter if working with live animals: once the uterus is removed, the animal would have to be sacrificed.

Activity 5: Hormone Replacement Therapy

1. Click on the syringe, drag it to the bottle of **saline**, and release the mouse button. The syringe will automatically fill with 1 ml of saline.
2. Drag the syringe to the **Control** rat and place the tip of the needle in the rat's lower abdominal area. Injections into this area are considered *interperitoneal* and will quickly be picked up by the abdominal blood vessels. Release the mouse button—the syringe will empty into the rat and automatically return to its holder. Click **Clean** on the syringe holder to “clean” the syringe of all residue.
3. Click on the syringe again, this time dragging it to the bottle of **estrogen**, and release the mouse button. The syringe will automatically fill with 1 ml of estrogen.
4. Drag the syringe to the **Experimental** rat and place the tip of the needle in the rat's lower abdominal area. Release the mouse button—the syringe will empty into the rat and automatically return to its holder. Click **Clean** on the syringe holder to “clean” the syringe of all residue.
5. Click the **Clock** above the **Elapsed Days** display. You will notice the hands sweep the clock face twice, indicating that 24 hours have passed.
6. Repeat steps 1–5 until each rat has received a total of 7 injections over the course of 7 days (1 injection per day). Note that the **# of injections** display below each rat cage records how many injections the rat has received. The control rat should receive 7 injections of saline, whereas the experimental rat should receive 7 injections of estrogen.
7. Next, click on the box of weighing paper. You will see a small piece of paper appear. Click and drag this paper over to the top of the scale and release the mouse button.
8. Notice that the scale will give you a weight for the paper. With the mouse arrow, click on the **Tare** button to tare the scales to zero (0.00 gms), adjusting for the weight of the paper.

9. You are now ready to remove the uteruses. In a wet lab, this would require surgery. Here you will simply click on the **Remove Uterus** button found in each rat cage. The rats will disappear, and a uterus (consisting of a uterine body and two uterine horns) will appear in each cage.

10. Click and drag the uterus from the Control rat over to the scale and release it on the weighing paper. Click on the **Weigh** button to obtain the weight. Record the weight here:

Uterus weight (Control): _____ gms

11. Click **Record Data**.

12. Click **Clean** on the weight scale to dispense of the weighing paper and uterus.

13. Repeat steps 7 and 8. Then click and drag the uterus from the Experimental rat over to the scale and release it on the weighing paper. Click **Weigh** to obtain the weight. Record the weight here:

Uterus weight (Experimental): _____ gms

14. Click **Record Data**.

15. Click **Clean** on the weight scale to dispense of the weighing paper and uterus.

16. Click **Tools** → **Print Data** to print your data.

How does the Control uterus weight compare to the Experimental uterus weight?

What can you conclude about the administration of estrogen injections on the experimental animal?

What might be the effect if testosterone had been administered instead of estrogen? Explain your answer.

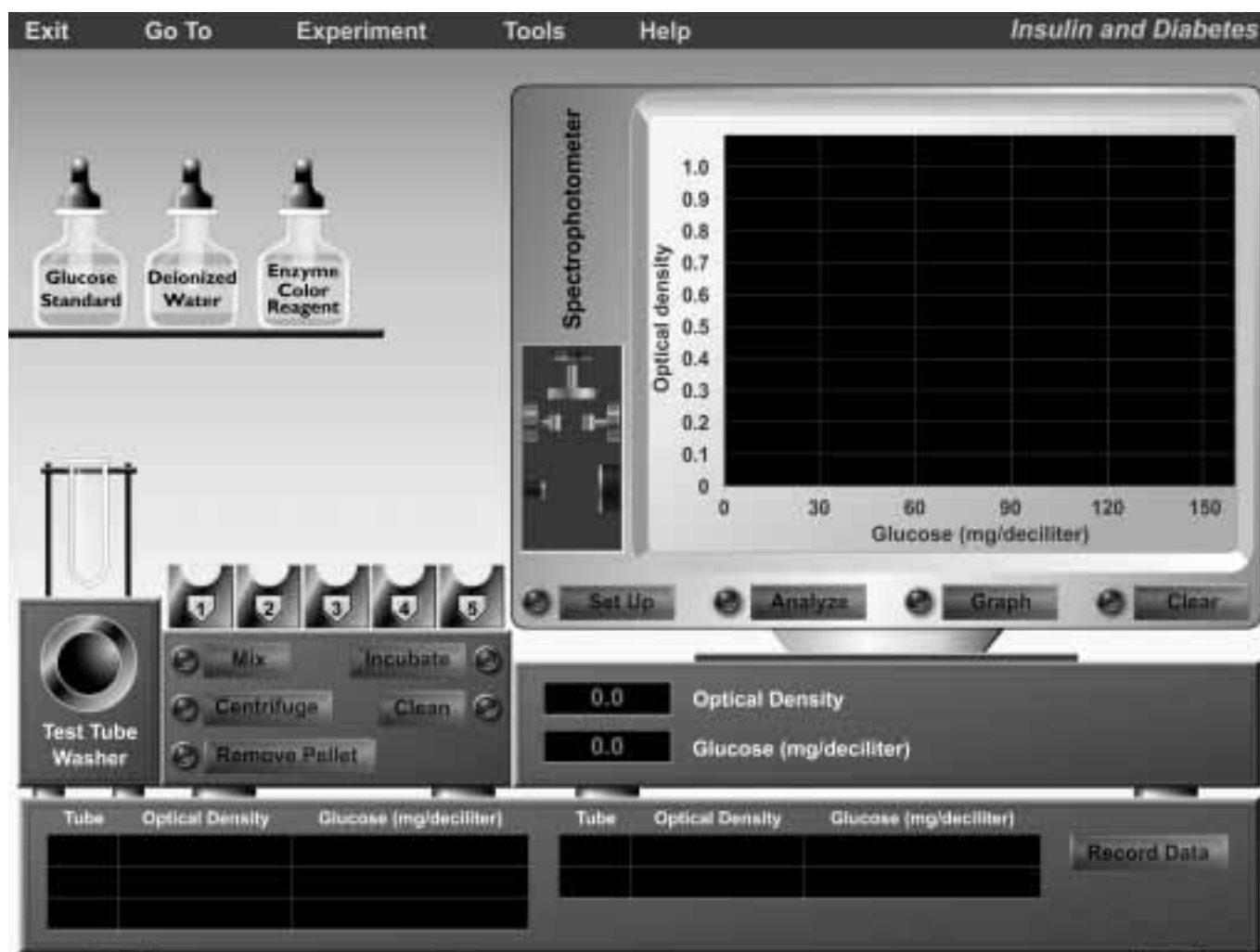


Figure 4.3 Opening screen of the Insulin and Diabetes experiment, Part 1.

Insulin and Diabetes

Insulin is produced by the β -cells of the endocrine portion of the pancreas. It is vital to the regulation of blood glucose levels because it enables the body's cells to absorb glucose from the bloodstream. Glucose absorbed from the blood can enter cells (usually liver or muscle cells), where excess glucose is used to form glycogen (animal starch). It is estimated that 75% of glucose taken in with a meal is stored in this manner. Since humans are considered "discontinuous feeders," this production of animal starch after a meal ensures that a glucose supply will be available for several hours after intake. The body has to maintain a certain level of glucose in the blood to serve nervous system cells, into which only glucose can be absorbed. When glucose levels in the blood fall below a certain point, the α cells of the pancreas then produce **glucagon**. The job of this hormone is to break the stored glycogen down into glucose to be released into the blood.

When insulin is not produced by the pancreas, **diabetes mellitus Type I** results. When insulin *is* produced by the pancreas but the body fails to respond to it, **diabetes mellitus**

Type II results. In either case, glucose remains in the bloodstream, unable to be taken up by the body's cells to serve as the primary fuel for metabolism. Excess glucose in the blood is then filtered by the kidney. Since the re-uptake of filtered glucose involves a finite number of receptors in kidney cells, some excess glucose will not be re-absorbed into the body and will instead pass out of the body in urine. The lack of insulin for glucose transport also affects muscle, and results in muscle cells undergoing protein catabolism so that the freed amino acids can form glucose within the liver. This action puts the body into a negative nitrogen balance from the resulting protein depletion and tissue wasting. Also associated with this condition is poor resistance to infections.

In the following experiment you will be studying the effects of insulin treatment for diabetes Type I. The experiment is divided into two parts. In Part I you will be obtaining a **glucose standard curve**, which will be explained shortly. In Part II you will compare the glucose levels of a normal rat to those of a diabetic rat, and then compare them again after each rat has been injected with insulin.

Part I

Activity 6: Obtaining a Glucose Standard Curve

To begin, select **Insulin and Diabetes—Part 1** from the **Experiments** menu (Figure 4.3). Select **Balloons On** from the Help menu for help identifying the equipment onscreen (you will see labels appear as you roll over each piece of equipment). Select **Balloons Off** to turn this feature off before you begin the experiments.

On the right side of the opening screen is a special **spectrophotometer**. The spectrophotometer is one of the most widely used research instruments in biology. It is used to measure the amounts of light of different wavelengths absorbed and transmitted by a pigmented solution. Inside of the spectrophotometer is a source of white light, which is separated into various wavelengths (or colors) by a prism. The user selects a wavelength (color), and light of this color is passed through a tube, or *cuvette*, containing the sample being tested. (For this experiment, the spectrophotometer light source will be pre-set for a wavelength of 450 nm.) The light transmitted by the sample then passes onto a photoelectric tube, which converts the light energy into an electrical current. The current is then measured by a meter. Alternatively, the light may be measured before the sample is put into the light path, and the amount of light absorbed—called **optical density**—may then be measured. Using either method, the change in light transmittance or light absorbed can be used to measure the amount of a given substance in the sample being tested.

In Part II you will be using the spectrophotometer to determine how much glucose is present in blood samples that you will be taking from two rats. But before you can do that, you must first obtain a **glucose standard curve** so that you have a point of reference for converting optical density readings into glucose readings (which will be measured in mg/deciliter). To do this you will prepare five test tubes that contain known amounts of glucose: 30 mg/deciliter, 60 mg/deciliter, 90 mg/deciliter, 120 mg/deciliter, and 150 mg/deciliter, respectively. You will then use the spectrophotometer to determine the corresponding optical density readings for each of these known amounts of glucose. You will then use this information to perform Part II.

Also on the screen are three dropper bottles, a test tube washer, a test tube dispenser (on top of the washer), and a test tube incubation unit that you will need to prepare the samples for analysis.

1. Click and drag the test tube (on top of the test tube washer) into slot 1 of the incubation unit. You will see another test tube pop up from the dispenser. Click and drag this second test tube into slot 2 of the incubation unit. Repeat until you have dragged a total of five test tubes into the five slots in the incubation unit.

2. Click and hold the mouse button on the dropper cap of the **Glucose Standard** bottle. Drag the dropper cap over to tube #1. Release the mouse button to dispense the glucose. You will see that one drop of glucose solution is dropped into

the tube and that the dropper cap automatically returns to the bottle of glucose standard.

3. Repeat step 2 with the remaining four tubes. Notice that each subsequent tube will automatically receive one additional drop of glucose standard into the tube (that is, tube #2 will receive two drops, tube #3 will receive three drops, tube #4 will receive four drops, and tube #5 will receive 5 drops).

4. Click and hold the mouse button on the dropper cap of the **Deionized Water** bottle. Drag the dropper cap over to tube #1. Release the mouse button to dispense the water. Notice that four drops of water are automatically added to the first tube.

5. Repeat step 4 with tubes #2, 3, and 4. Notice that each subsequent tube will receive one *less* drop of water than the previous tube (that is, tube #2 will receive three drops, tube #3 will receive two drops, and tube #4 will receive one drop). Tube #5 will receive no drops of water.

6. Click on the **Mix** button of the incubator to mix the contents of the tubes.

7. Click on the **Centrifuge** button. The tubes will descend into the incubator and be centrifuged. When tubes are centrifuged, they are spun around a center point at high speed so that any particulate matter within the tube will settle at the bottom of the tube, forming what is called a “pellet.”

8. When the tubes resurface, click on the **Remove Pellet** button. Any pellets from the centrifuging process will be removed from the test tubes.

9. Click and hold the mouse button on the dropper cap of the **Enzyme-Color Reagent** bottle. Still holding the mouse button down, drag the dropper cap over to tube #1. When you release the mouse, you will note that five drops of reagent are added to the tube and that the stopper is returned to its bottle.

10. Repeat step 9 for the remaining tubes.

11. Now click **Incubate**. The tubes will descend into the incubator, where they will be shaken to completely mix the color reagent in the tube, incubate, and then resurface.

12. Using the mouse, click on **Set Up** on the spectrophotometer. This will warm up the instrument and get it ready for your readings. In this case, “set up” also includes setting the “zero” point so the spectrophotometer will accurately read the quantity of material contained in each tube.

13. Click and drag tube #1 into the spectrophotometer (right above the **Set Up** button) and release the mouse button. The tube will lock into place.

14. Click **Analyze**. You will see a spot appear on the screen, and values will appear in the **Optical Density** and **Glucose** displays.

15. Click **Record Data** on the data collection unit.

16. Click and drag the tube into the test tube washer.

17. Repeat steps 13–16 for the remaining test tubes.

18. When all five tubes have been analyzed, click on the **Graph** button. This is the glucose standard graph which you will use in Part II of the experiment. ■

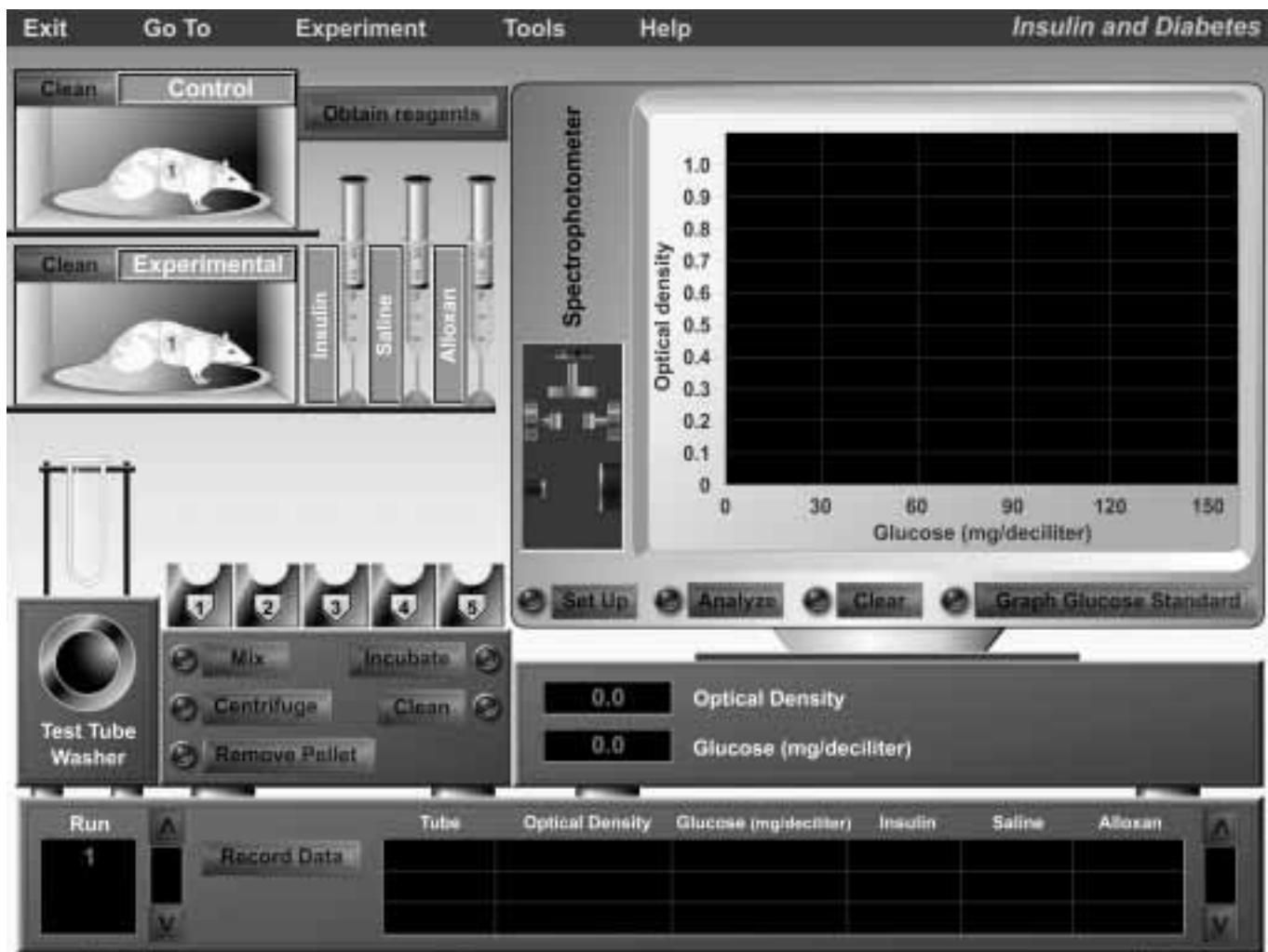


Figure 4.4 Opening screen of the Insulin and Diabetes experiment, Part 2.

Part II

Activity 7:

Comparing Glucose Levels Before and After Insulin Injection

Select **Insulin and Diabetes Part 2** from the **Experiment** menu.

The opening screen will look similar to the screen from Part I (Figure 4.4). Notice the two rats in their cages. One will be your control animal, the other will be your experimental animal. Also note the three syringes, containing insulin, saline, and alloxan, respectively. **Alloxan** is a drug that, when administered to an animal, selectively kills all the pancreatic β -cells that produce insulin, and renders the animal instantly diabetic.

In this experiment you will inject the control rat with saline and the experimental rat with alloxan. (Normally, injections are given *daily* for a week. In this simulation we will administer the injections only once, but we can see results as though the injections had been given over a longer period of time.)

After administering the saline and alloxan injections, you will obtain blood samples from the two rats. You will then inject both rats with insulin, and obtain blood samples again. Finally, you will analyze all the blood samples in the spectrophotometer (described in Part I) to compare the amounts of glucose present in the samples.

1. Click and drag the **Saline** syringe to the **Control** rat and release the mouse button to inject the animal.
2. Click and drag the **Alloxan** syringe to the **Experimental** rat and release the mouse button to inject the animal.
3. Click and drag a new test tube (from the test tube dispenser) over to the tail of the **Control** rat and release the mouse button. You will note three drops of blood being drawn from the tail into the tube. Next, click and drag the tube into test tube holder #1 in the incubator. (Note: The tail is a popular place to get blood from a rat. The end of the tail can easily be clipped and blood collected without really disturbing the rat. The tail heals quickly, with no harm to the animal.)
4. Click and drag another new test tube (from the test tube dispenser) over to the tail of the **Experimental** rat and release

the mouse button. Again, you will note that three drops of blood are drawn from the tail into the tube. Click and drag the tube into test tube holder #2 in the incubator.

5. Click and drag the **Insulin** syringe to the **Control** rat and release the mouse button to inject the animal.

6. Repeat step 5 with the **Experimental** rat.

7. Repeat steps 3 and 4 again, drawing blood samples from each rat and placing the samples into test tube holders #3 and #4.

8. Click the **Obtain reagents** button on the cabinet that currently displays the syringes. Both the syringes and rats will disappear, and you will see four dropper bottles in their place.

9. Click and hold the mouse button on the dropper of the **Deionized Water** bottle. Drag the dropper cap over to tube #1. Release the mouse button to dispense. You will note that five drops of water are added to the tube. This water is added so that all tubes will have the same volume.

10. Repeat step 9 for the remaining test tubes.

11. Click and hold the mouse button on the dropper of **Barium Hydroxide**. Drag the dropper cap over to tube #1. Release the mouse button to dispense. You will note that five drops of solution are added to the tube. (Barium hydroxide is used for clearing proteins and cells so that clear glucose readings may be obtained.)

12. Repeat step 11 for the remaining test tubes.

13. Click and hold the mouse button on the dropper of the **Heparin** bottle. Still holding the mouse button down, drag the dropper cap over to tube #1. Release the mouse button to dispense. Heparin is an anticoagulant that prevents the blood from clotting while being worked on.

14. Repeat step 13 for the remaining test tubes.

15. Click on the **Mix** button of the incubator to mix the contents of the tubes.

16. Click on the **Centrifuge** button. The tubes will descend into the incubator to be centrifuged and will then resurface.

17. Click on the **Remove Pellet** button to remove any pellets from the centrifuging process.

18. Click and hold the mouse button on the dropper of the **Enzyme Color Reagent** bottle. Drag the dropper cap to tube #1. Release the mouse to dispense.

19. Repeat step 18 with the remaining test tubes. In an actual wet lab, you would also shake the test tubes after adding the enzyme color reagent.

20. Click **Incubate** one more time. The tubes will descend into the incubator, incubate, and then resurface.

21. Click on **Set Up** on the spectrophotometer. This will warm up the instrument and get it ready for your readings.

22. Click **Graph Glucose Standard**. The graph from Part I of the experiment will appear on the monitor.

23. Click and drag tube #1 to the spectrophotometer and release the mouse button. The tube will lock into place.

24. Click **Analyze**. You will see a horizontal line appear on the screen and a value appear in the **Optical Density** display.

25. Drag the **moveable rule** (the red vertical line on the far right of the spectrophotometer monitor) over to where the horizontal line (from step 24) crosses the glucose standard line. Notice what happens to the **Glucose** display as you move the moveable rule to the left.

What is the glucose reading for where the horizontal line crosses the glucose standard line?

Test tube #1: _____ mg/deciliter glucose

This is your glucose reading for the sample being tested.

26. Click **Record Data** on the data collection unit.

27. Click and drag the test tube from the spectrophotometer into the test tube washer, then click **Clear** beneath the oscilloscope display.

28. Repeat steps 22–27 for the remaining test tubes. Record your glucose readings for each test tube here:

Test tube #2: _____ mg/deciliter glucose

Test tube #3: _____ mg/deciliter glucose

Test tube #4: _____ mg/deciliter glucose

How does the glucose level in test tube #1 compare to the level in test tube #2? Recall that tube #1 contains a sample from your control rat (which received injections of saline), and that tube #2 contains a sample from your experimental rat (which received injections of alloxan).

Provide an explanation for this result:

What is the condition that alloxan has caused in the experimental rat?

How does the glucose level in test tube #3 compare to the level in test tube #1?

Provide an explanation for this result:

How does the glucose level in test tube #4 compare to the level in test tube #2?

Provide an explanation for this result:

What was the effect of administering insulin to the control animal?

What was the effect of administering insulin to the experimental animal?

Click **Tools** → **Print Data** to print your recorded data. ■

Histology Review Supplement

Turn to p. 154 for a review of endocrine tissue.