

Endocrine System Physiology

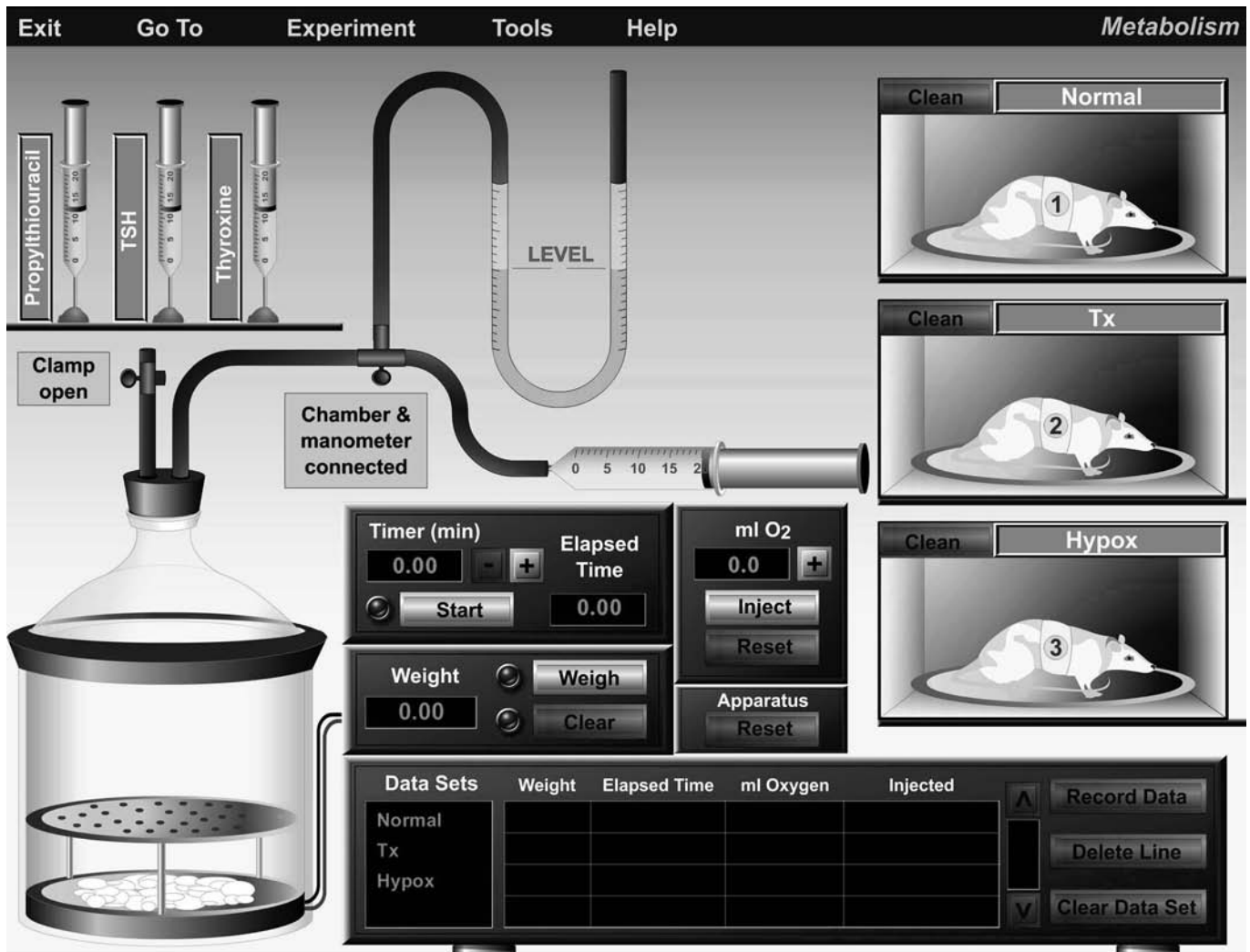
OBJECTIVES

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 bone density
8. To explain how hormone replacement therapy works
9. To explain how fasting plasma glucose is used to diagnose diabetes
10. To understand how levels of cortisol and ACTH can be used to diagnose endocrine diseases

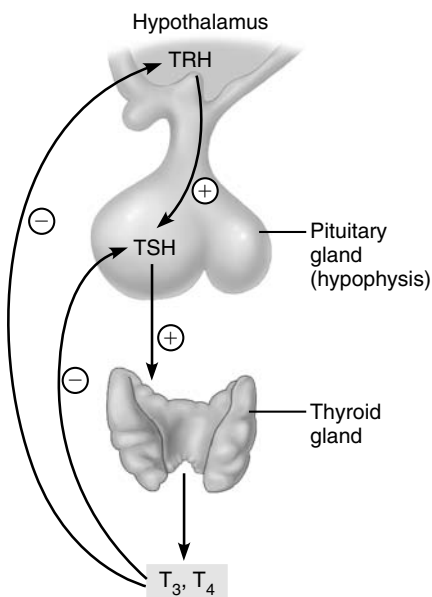
The endocrine system regulates the functioning of every cell, tissue, and organ in the body. It acts to maintain a stable internal body environment, regardless of changes occurring within or outside of the body. Endocrine cells have the ability to sense and respond to changes via the excretion of specific 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



(a)



(b)

FIGURE 4.1 Metabolism and the thyroid gland. (a) Opening screen of the Metabolism experiment. (b) The regulation of thyroid secretion. + indicates stimulation of release, - indicates inhibition of release, T₃ = triiodothyronine, T₄ = thyroxine, TRH = thyrotropin-releasing hormone, TSH = thyroid-stimulating hormone.

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.

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 into 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.

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 drop-down menu, select **Exercise 4: Endocrine System Physiology** and click **GO**. Before you perform the activities watch the **BMR Measurement** video to see an experiment in which basal metabolic rate is measured. Then click **Metabolism**. The opening screen will appear in a few seconds (see Figure 4.1). Select **Balloons On/Off** from the Help menu for help identifying the equipment on-screen (you will see labels appear as you roll over each piece of equipment). Select **Balloons On/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 tubing). 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. 45). 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.

1. 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.
2. 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.
3. Be sure the indicator next to the T-connector reads "Chamber and manometer connected." If not, click on the **T-connector knob**.
4. 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."
5. Click the (+) button on the Timer so that the Timer display reads 1.00.
6. 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.
7. 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.
8. 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."
9. Click the clamp to open it so that the rat can once again breathe outside air.
10. Look at the difference between the level in the left and right arms of the U-tube and estimate the volume of O₂ that will need to be injected by counting the divider lines on both sides. Then click the (+) button under the ml O₂ until the display reads that number. Then click **Inject** and watch what happens to the fluid in the two arms. When the volume is equalized the word "Level" will appear and stay on the screen. If you are under, click the (+) and then **Inject**. If you

are over the word "Level" will flash and then disappear. You will then have to click the **Reset** button and try a lower volume. (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."

11. 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."

12. 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."

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

Which rat had the fastest baseline metabolic rate?

Why did the metabolic rates differ?

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

Chart 1			
	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.

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. Choose a rat to test. You will eventually test all three, and it doesn’t matter what order you test them in. Do not drag the rat to the chamber yet. Under **Data Sets**, the simulation will 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 drug that inhibits the production of thyroxine by blocking the attachment of iodine to tyrosine residues and interfering with the conversion of thyroxine with triiodothyronine.

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

1. Under **Data Sets**, the simulation will 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

Follicle-stimulating hormone (FSH) stimulates ovarian follicle growth. While the follicles are developing, they produce the hormone *estrogen*. As the female enters menopause, the ovaries stop producing estrogen. One of the symptoms of menopause is loss of bone density, which can result in osteoporosis and bone fractures. Postmenopausal treatments to prevent osteoporosis include the administration of estrogen to increase bone density. Calcitonin is a hormone that inhibits osteoclast activity and stimulates calcium uptake for deposit in bone.

In this experiment we will use three ovariectomized rats because they are no longer producing estrogen due to the removal of their ovaries. The three rats were chosen because each has a baseline T score of -2.6 , indicating osteoporosis.

T scores are interpreted as follows: normal = $+1$ to -0.99 ; osteopenia (bone thinning) = -1.0 to -2.49 ; osteoporosis = -2.5 and below. You will administer either estrogen therapy or calcitonin therapy, two types of **hormone replacement therapy**. The third rat will serve as an untreated control and receive daily injections of saline. The vertebral bone density (VBD) of each rat will be measured with dual X-ray absorptiometry (DXA) to obtain the T score.

Start by selecting **Hormone Replacement Therapy** from the **Experiment** menu. A new screen will appear (Figure 4.2) with three ovariectomized rats in cages. (Note that if this were a wet lab, the ovariectomies would have been performed on the rats a month 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 bottle of calcitonin, a clock, and a dual X-ray absorptiometry bone density scanner.

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. Click and hold the syringe and 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 *intraperitoneal* 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. Click and hold the syringe, drag it to the **estrogen-treated** 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 on the syringe again, this time dragging it to the bottle of **calcitonin**, and release the mouse button. The syringe will automatically fill with 1 ml of calcitonin.
6. Click and hold the syringe, drag it to the **calcitonin-treated** 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.
7. Click on the **clock**. You will notice the hands sweep the clock face twice, indicating that 24 hours have passed.
8. Repeat steps 1–7 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** displayed below each rat cage records how many injections the rat has received. The control rat should receive 7 injections of saline, the estrogen-treated

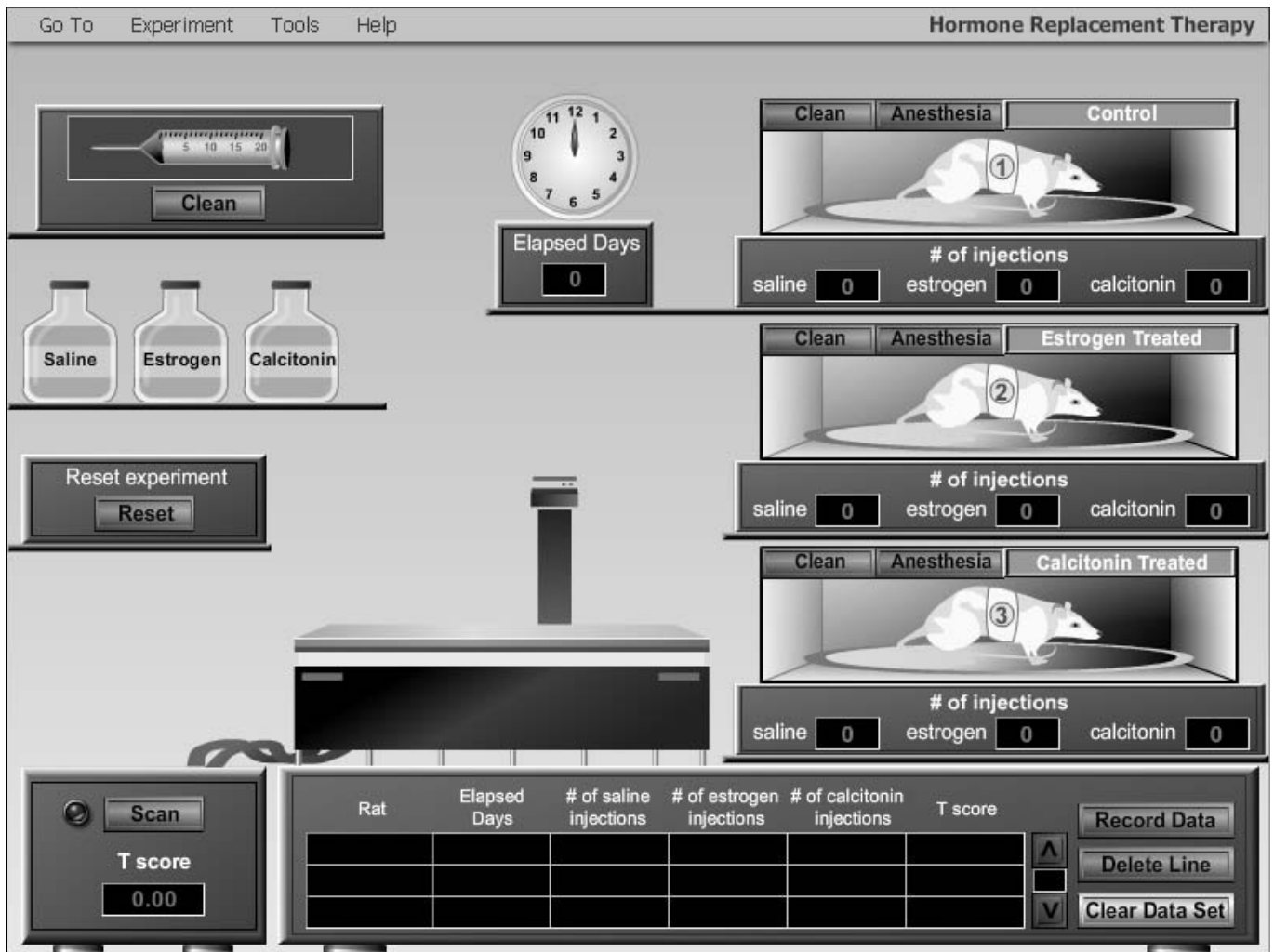


FIGURE 4.2 Opening screen of the Hormone Replacement Therapy experiment.

rat should receive 7 injections of estrogen, and the calcitonin-treated rat should receive 7 injections of calcitonin.

9. You are now ready to measure the effect of each of the solutions. First, predict the effect that each solution will have on the rat's vertebral bone density.

Saline injections _____

Estrogen injections _____

Calcitonin injections _____

10. A gaseous anesthetic will be applied to immobilize the rats for imaging. Click on the **Anesthesia** button for the control rat to immobilize the rat.

11. Click on the **control** rat and drag it to the exam table. Release the mouse to release the rat.

12. Click the **Scan** button to activate the scanner. Record the T score.

T score (control): _____

13. Click **Record Data**.

14. Click and drag the rat to return it to its cage.

15. Repeat steps 10–14 for the **estrogen-treated** rat.

T score (estrogen): _____

16. Repeat steps 10–14 for the **calcitonin-treated** rat.

T score (calcitonin): _____

17. Click **Tools** → **Print Data** to print your recorded data for this experiment.

Recall that the baseline value for all three rats was -2.6 . T scores are interpreted as follows: normal = $+1$ to -0.99 ; osteopenia (bone thinning) = -1.0 to -2.49 ; osteoporosis = -2.5 and below.

What effect did the administration of estrogen injections have on the estrogen-treated rat?

What effect did the administration of calcitonin injections have on the calcitonin-treated rat?

How did these results compare with your predictions?

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.

The following 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 use the standard curve to measure fasting plasma glucose levels in patients to diagnose diabetes mellitus.

Part I

ACTIVITY 6

Obtaining a Glucose Standard Curve

To begin, select **Insulin and Diabetes—Part 1** from the **Experiment** menu (Figure 4.3). Select **Balloons On/Off** from the Help menu for help identifying the equipment on-screen (you will see labels appear as you roll over each piece of equipment). Select **Balloons On/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.

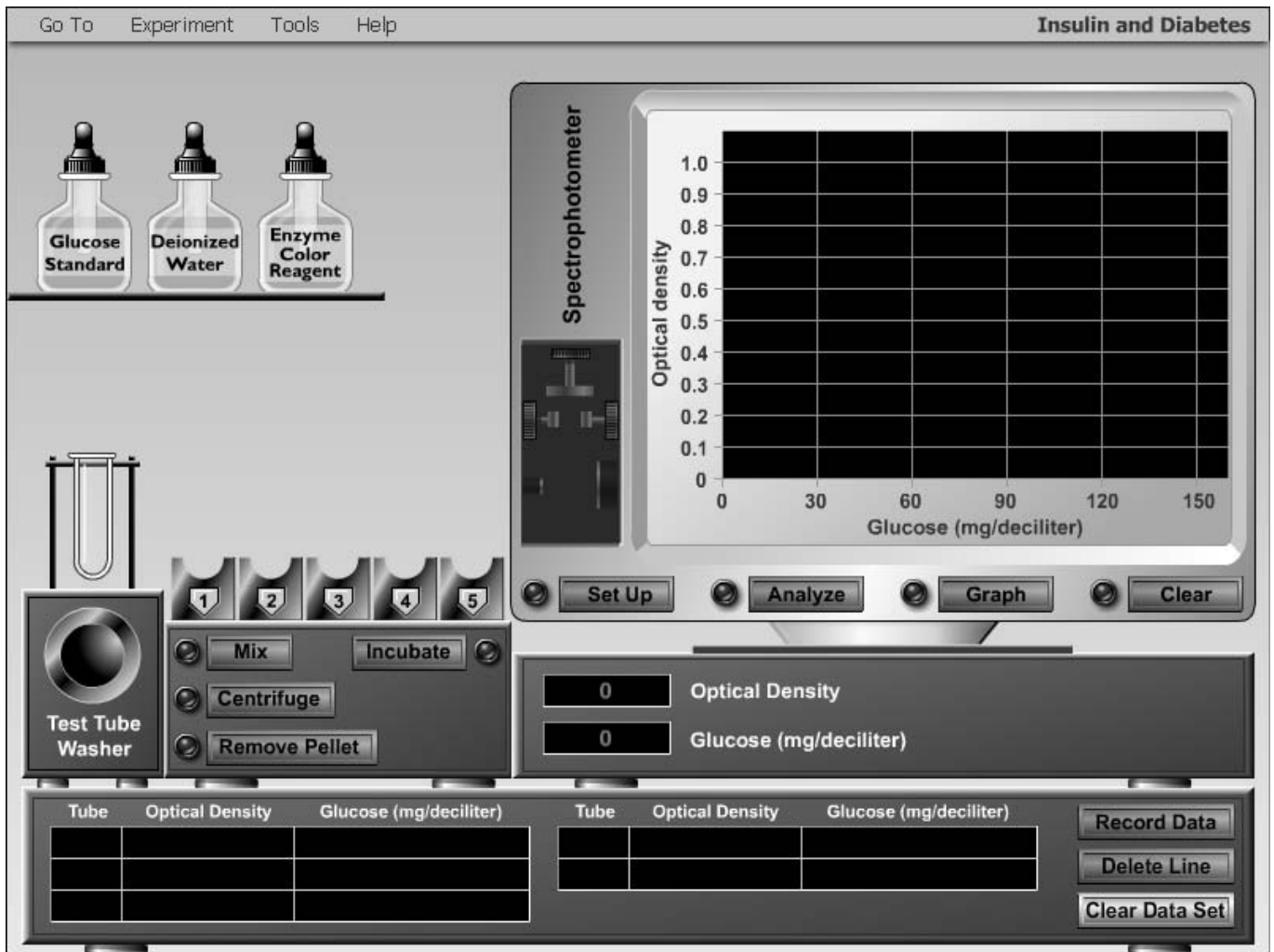


FIGURE 4.3 Opening screen of the Insulin and Diabetes experiment, Part 1.

You will see that one drop of glucose solution is dropped into the tube.

3. The dropper will automatically slide over to each of the remaining samples. 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. The dropper will automatically slide over to each of the remaining samples. 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.

10. The dropper will automatically slide over to each of the remaining samples.

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 (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. ■

Part II

ACTIVITY 7

Measuring Fasting Plasma Glucose

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

A new screen will appear (Figure 4.4a). Two reagents in addition to those used in Part I are present, as well as five patient samples. To undergo the fasting plasma glucose (FPG) test, patients must fast for a minimum of 8 hours prior to the blood draw. Plasma samples will be measured in the spectrophotometer, and the glucose standard curve generated in Part I will be used to determine fasting plasma glucose levels in the five patient samples. A patient with two separate FPG tests greater than or equal to 126 mg/dl is diagnosed with diabetes. FPG values between 110 and 126 mg/dl are indicative of impairment or borderline impairment of glucose uptake by cells. FPG values less than 110 mg/dl are normal.

If the FPG is borderline, another test, the oral glucose tolerance test (OGTT), is performed. In this test, the patient also fasts for 8 hours. The patient then ingests a concentrated glucose solution, and blood is drawn and tested at periodic intervals. Glucose and sometimes insulin levels are measured. The 2-hour glucose level should be below 140 mg/dl. A 2-hour OGTT level between 140 and 200 mg/dl indicates impaired glucose tolerance, and a level above 200 mg/dl confirms the diabetes diagnosis. Individuals with impaired fasting glucose values and impaired glucose tolerance are at a higher risk of developing type 2 diabetes. If a patient is pregnant, an FPG value greater than 110 mg/dl could indicate gestational diabetes and a strict diet should be followed for the remainder of the pregnancy.

1. Click and drag a 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 the 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 **Sample 1** and then drag the dropper to the first test tube. The dropper will automatically dispense 3 drops of blood into the test tube and automatically return to the vial.

3. Repeat step 2 for the remaining patient samples.

4. Click and hold the mouse button on the dropper cap of the **deionized water** bottle. Drag the dropper cap over to test tube 1. Release the mouse to dispense the water. Five drops of water will be dispensed into the tube.

5. The dropper will automatically slide over to the remaining tubes and will add five drops to each tube. The dropper will automatically return to the vial when the dispensing is complete.

6. Click and hold the mouse button on the dropper of **barium hydroxide**. Drag the dropper cap over to test tube 1. Release the mouse button to dispense the barium hydroxide. Five drops of the solution will be dispensed.

7. The dropper will automatically slide over to the remaining tubes and will add five drops to each tube. The dropper will automatically return to the vial when the dispensing is complete. (Barium hydroxide is used for clearing proteins and cells so that clear glucose readings may be obtained.)

8. Click and hold the mouse button on the dropper of the **heparin** bottle. Drag the dropper cap over to tube 1. Release the mouse button to dispense the heparin.

9. The dropper will automatically slide over to the remaining tubes and will add one drop to each tube. The dropper will automatically return to the vial when the dispensing is complete. (Heparin is an anticoagulant that prevents blood clotting.)

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

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

12. Click on the **Remove Pellet** button to remove any pellets from the centrifugation process.

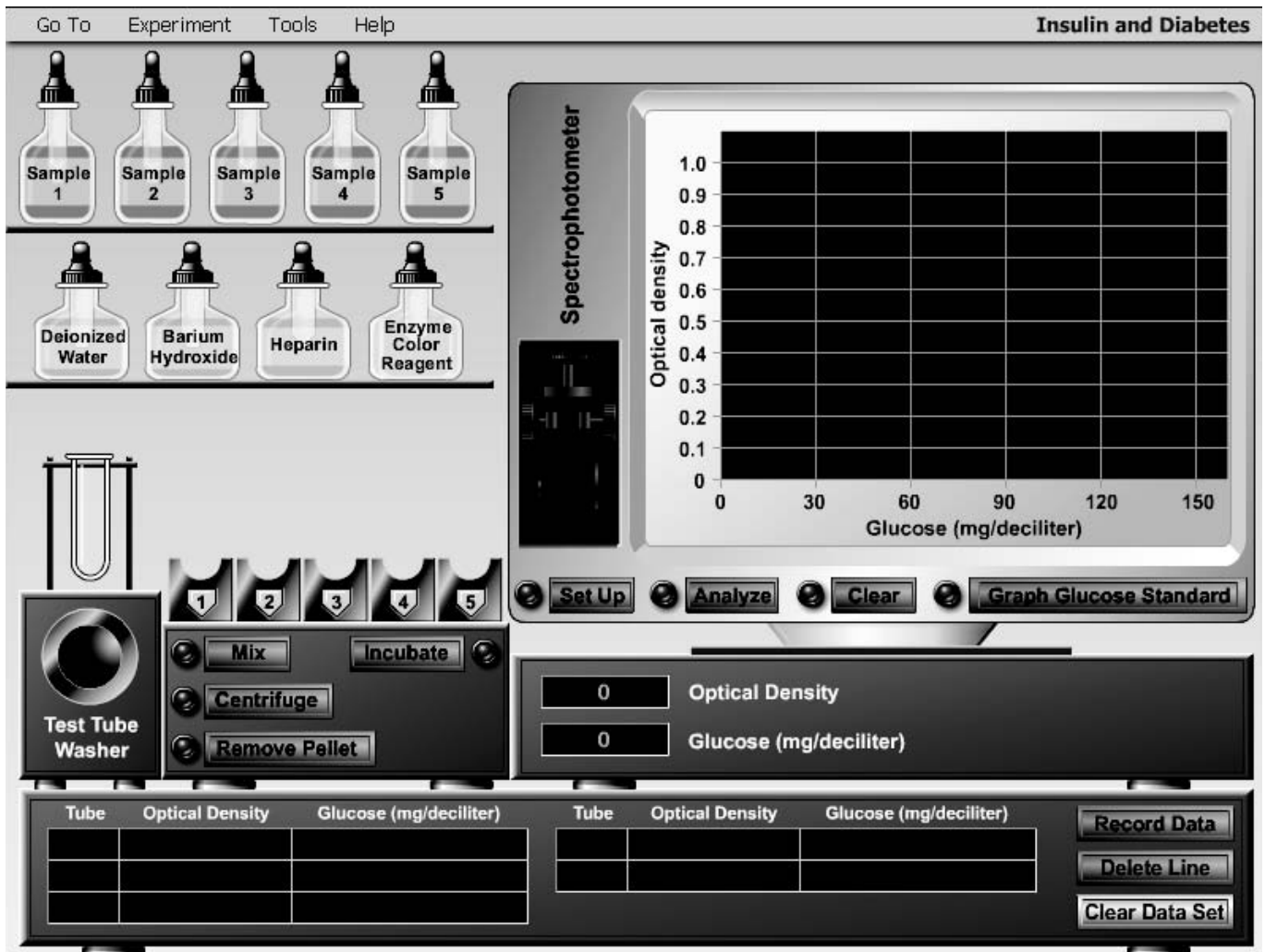
13. Click and hold the mouse button on the dropper of the **enzyme color reagent** bottle. Drag the dropper cap to test tube 1. Release the mouse to dispense the reagent.

14. The dropper will automatically slide over to the remaining tubes and will add five drops to each tube. The dropper will automatically return to the vial when the dispensing is complete.

15. Click **Incubate**. The tubes will descend into the incubator, incubate, and then resurface.

16. Click **Set Up** on the spectrophotometer to warm up the instrument and get it ready for your readings.

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



(a)

FIGURE 4.4 Insulin and diabetes. (a) Opening screen of the Insulin and Diabetes experiment, Part 2.

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

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

20. Drag the movable ruler (the vertical line on the far right of the spectrophotometer monitor) over to where the horizontal line (from step 19) crosses the glucose standard line. Watch what happens to the **Glucose** display as you move the movable ruler to the left.

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

Sample 1: glucose concentration of _____ mg/deciliter

This is your glucose reading for the patient being tested.

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

22. Click and drag the test tube from the spectrophotometer into the test tube washer, then click **Clear** under the display.

23. Repeat steps 17–22 for the remaining test tubes. Record your glucose readings for each test tube here:

Sample 2: glucose concentration of _____ mg/deciliter

Sample 3: glucose concentration of _____ mg/deciliter

Sample 4: glucose concentration of _____ mg/deciliter

Sample 5: glucose concentration of _____ mg/deciliter

For which patient(s) were the glucose reading(s) in the normal range?

For which patient(s) were the fasting plasma glucose reading(s) in the diabetic range?

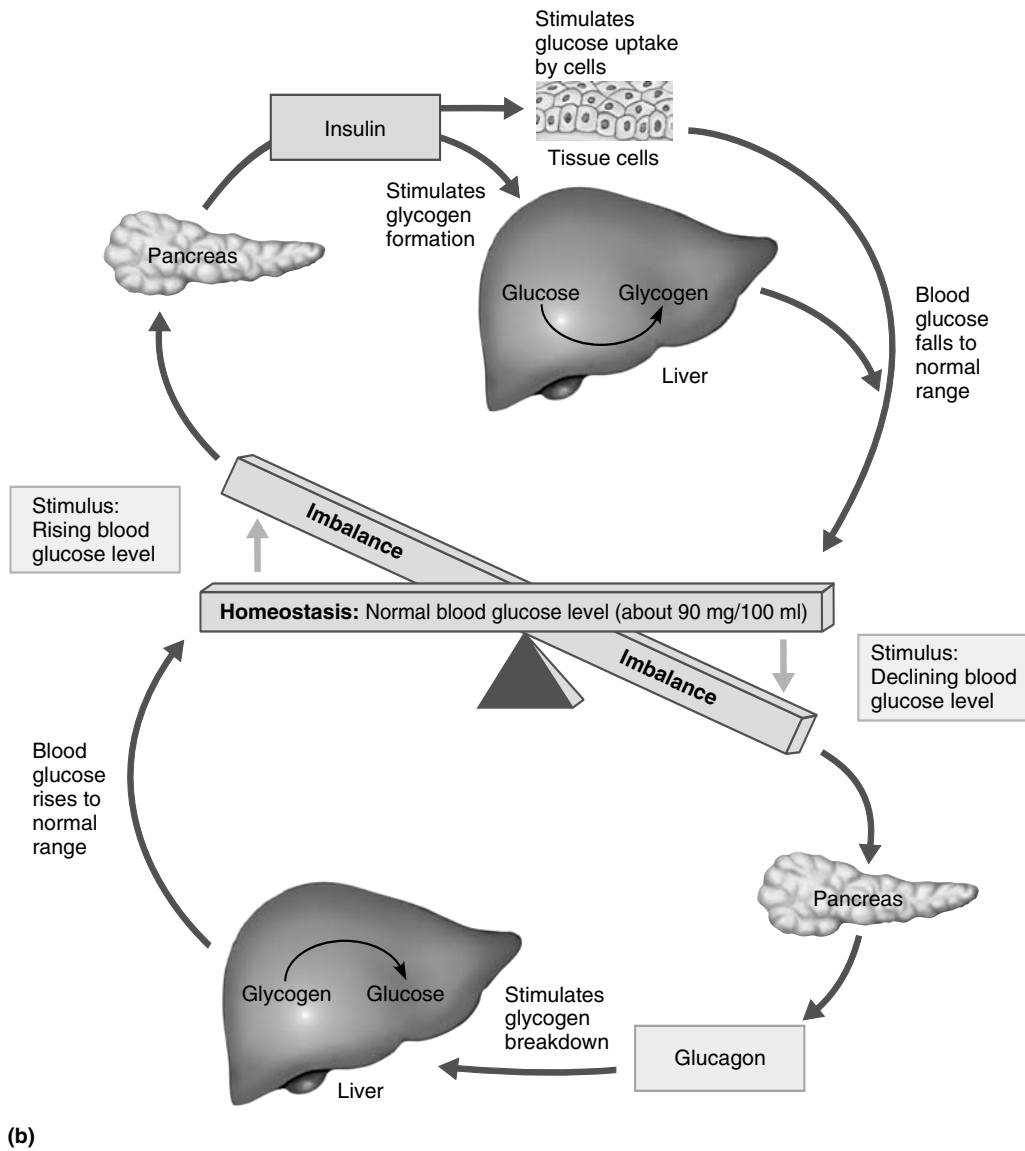


FIGURE 4.4 (continued) Insulin and diabetes. (b) Regulation of blood sugar levels by insulin and glucagon.

For which patient(s) were the fasting plasma glucose reading(s) in the impaired range?

What recommendations would you make to a patient with an impaired FPG value who also tested in the impaired range with the oral glucose tolerance test?

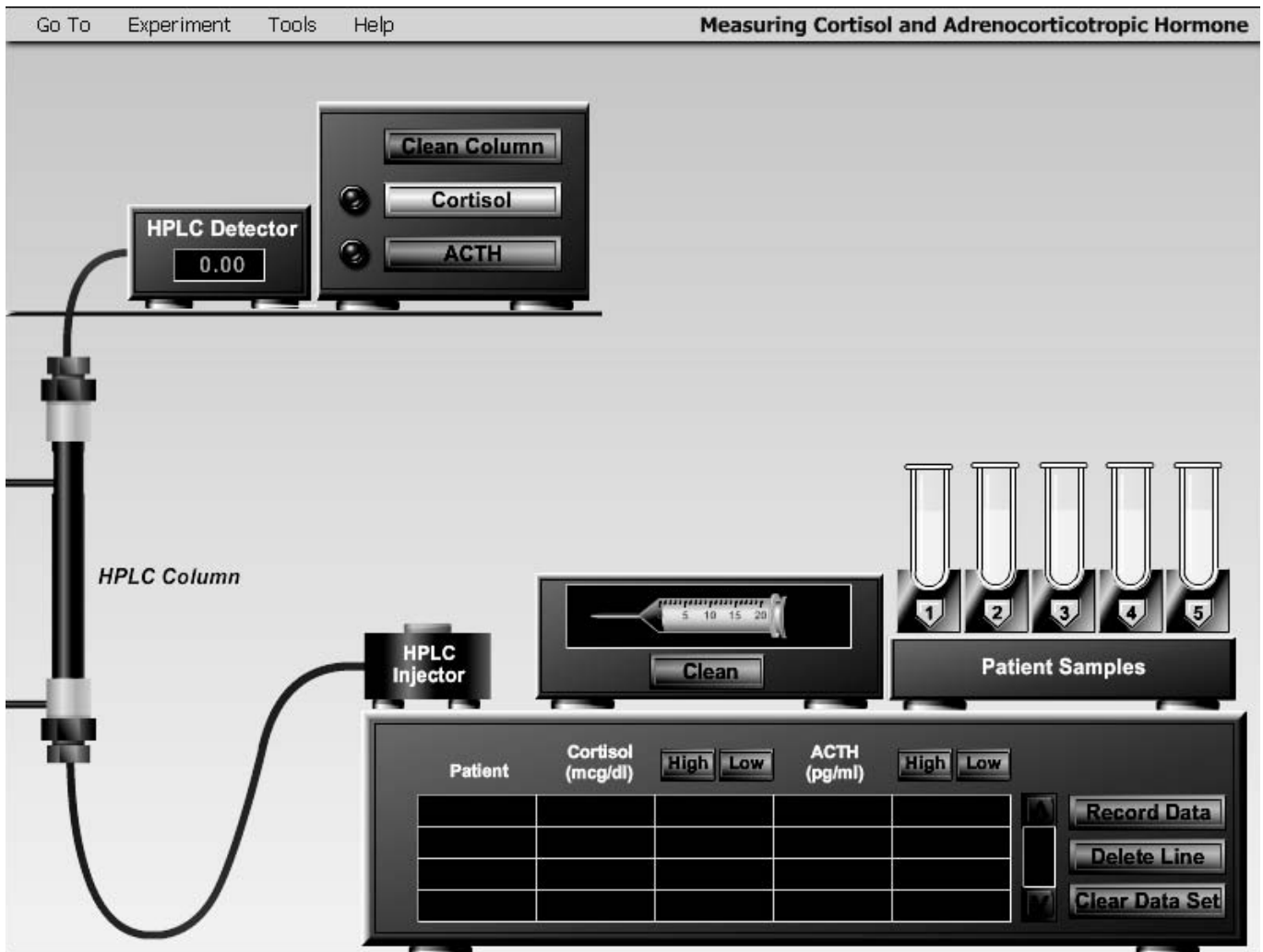
Patient 3 is pregnant; how might this change the diagnosis? What recommendations would you make to this patient?

ACTIVITY 8

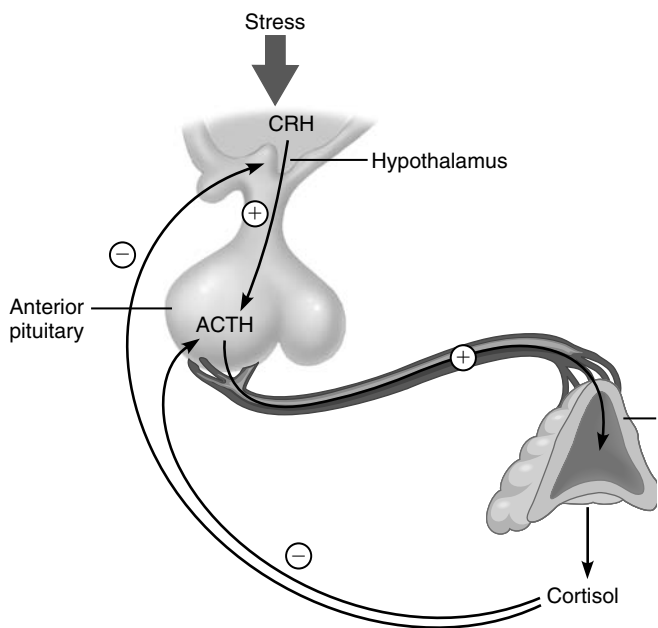
Measuring Cortisol and Adrenocorticotropic Hormone

Cortisol, a hormone secreted by the adrenal cortex, is key to the long-term regulation of stress. Cortisol release is stimulated by adrenocorticotropic hormone (ACTH), a hormone released by the anterior pituitary. ACTH release is stimulated by a hypothalamic hormone, corticotropin-releasing hormone (CRH). Increased levels of cortisol negatively feed back to inhibit the release of both ACTH and CRH. See Figure 4.5 for the regulation of cortisol secretion.

Increased cortisol in the blood, or hypercortisolism, is referred to as Cushing’s syndrome if it is due to an adrenal tumor. Hypercortisolism caused by a pituitary tumor also causes levels of ACTH to increase and is referred to as Cushing’s disease. Cushing’s syndrome can also be iatrogenic;



(a)



(b)

FIGURE 4.5 Following the cortisol release pathway. (a) Opening screen of the Measuring Cortisol and ACTH experiment. (b) The regulation of cortisol secretion. + indicates stimulation of release, - indicates inhibition of release, CRH = corticotropin-releasing hormone, ACTH = adrenocorticotropic hormone.

Table 4.1	Cortisol and ACTH Disorders	
	Cortisol Level	ACTH Level
Cushing's syndrome (primary hypercortisolism)	High	Low
Cushing's disease (secondary hypercortisolism)	High	High
Iatrogenic Cushing's syndrome	High	Low
Addison's disease (primary adrenal insufficiency)	Low	High
Secondary adrenal insufficiency (hypopituitarism)	Low	Low

that is, physician induced. This occurs when glucocorticoid hormones such as prednisone are administered for the treatment of rheumatoid arthritis, asthma, or lupus and is often referred to as “steroid diabetes” because it results in hyperglycemia.

Hypocortisolism can occur due to adrenal insufficiency. In primary adrenal insufficiency, also known as Addison's disease, the low cortisol is directly due to gradual destruction of the adrenal cortex, and ACTH levels are typically elevated as a compensatory effect. Secondary adrenal insufficiency also results in low levels of cortisol, usually due to damage to the pituitary gland. Levels of ACTH are also low in secondary adrenal insufficiency.

A variety of endocrine disorders are related to both high and low levels of cortisol and adrenocorticotrophic hormone. Table 4.1 summarizes these endocrine disorders.

Start by selecting **Measuring Cortisol and Adrenocorticotrophic Hormone** from the **Experiment** menu. A new screen will appear (Figure 4.5a) with five patient plasma samples and an HPLC (high-performance liquid chromatography) column that will be used to simulate the measurement of cortisol and adrenocorticotrophic hormone (ACTH). There is a syringe that will be used to inject the samples into the HPLC injector for analysis. The **Cortisol** and **ACTH** buttons are used to prepare the column with solvents used to separate the two different hormones. The detector will measure the amount of the hormone and convert it into a concentration value.

1. Start the experiment by clicking on the **Cortisol** button. This will prepare the column for the separation and measurement of cortisol.
2. Click and hold the syringe and drag it over to the first patient sample. Then release the mouse. The syringe will fill with plasma.
3. Click and hold the syringe again and drag it over to the **HPLC injector**. Then release the mouse. The sample will enter the tubing and flow through the column. The detector will display the concentration of cortisol in the first patient sample.

Table 4.2	Abnormal Morning Cortisol, ACTH Levels	
	High	Low
Cortisol	≥ 23 mcg/dl	< 5 mcg/dl
ACTH	≥ 80 pg/ml	< 20 pg/ml

Note: 1 mcg = 1 μ g = 1 microgram

4. Click **Record Data**.
5. Click the **Clean** button under the syringe to prepare it for the next sample.
6. Click the **Clean Column** button near the top of the screen to remove residual cortisol from the column.
7. Repeat steps 2–6 for the remaining four patient samples.
8. Next, prepare the column for ACTH separation and measurement by clicking on the **ACTH** button.
9. Click and hold the syringe and drag it over to the first patient sample. Then release the mouse. The syringe will fill with plasma.
10. Click and hold the syringe again and drag it over to the HPLC injector. Then release the mouse. The sample will enter the tubing and flow through the column. The detector will display the concentration of ACTH in the first patient sample.
11. Click **Record Data**.
12. Click the **Clean** button under the syringe to prepare it for the next sample.
13. Click the **Clean Column** button near the top of the screen.
14. Repeat steps 9–13 for the remaining four patient samples.
15. Select a row in the Data Set and choose **High** or **Low** based on the breakpoints shown in Table 4.2 for cortisol and ACTH in plasma from a morning blood draw.
16. Click **Tools** → **Print Data** to print your recorded data for this experiment.
17. Record your results for each patient here and circle High or Low:

Patient 1: Cortisol _____ mcg/dL High/Low

ACTH _____ pg/ml High/Low

Patient 2: Cortisol _____ mcg/dL High/Low

ACTH _____ pg/ml High/Low

Patient 3: Cortisol _____ mcg/dL High/Low

ACTH _____ pg/ml High/Low

Patient 4: Cortisol _____ mcg/dL High/Low

ACTH _____ pg/ml High/Low

Patient 5: Cortisol _____ mcg/dL High/Low

ACTH _____ pg/ml High/Low

Histology Review Supplement

For a review of endocrine tissue, go to **Exercise H: Histology Atlas & Review** on the **PhysioEx website** to print out the **Endocrine Tissue Review** worksheet.