The Endocrine System

I. Objectives

A. To become familiar with the structures, locations, and functions of the various endocrine glands.

B. To perform and interpret an experiment to examine the action of insulin and glucagon on a short-term basis.

C. To examine the effect of excess insulin on goldfish.

II. Glands of the Endocrine System

Endocrinology is the study of the structure and function of the ductless glands. During embryonic development, glands develop as epithelial surfaces fold inward. If the gland retains its opening to the surface (a surface which could be internal or external) then its secretions will be transported there through the connecting duct. Such a gland is called an exocrine gland. Examples are sweat glands, salivary glands, and mammary glands. However, if during development the gland loses its opening to the surface, then its secretions will be released to the bloodstream for transport. Such a gland is called a ductless or endocrine gland, and the secretions from such glands are called hormones. Examples are the thyroid and pituitary glands. Some organs have both an exocrine and an endocrine function. An example is the pancreas where the secretions that enter the small intestine are exocrine in nature. The endocrine part of the pancreas releases the hormones insulin and glucagon into the bloodstream for distribution to essentially all cells of the body.

The body has two main systems that coordinate activities of the various organs, the nervous system and the endocrine system. The nervous system produces rapid effects; endocrine responses are usually slower. Although some endocrine glands function independently, the nervous system probably influences and controls most endocrine functions. For example, the hypothalamus secretes releasing factors into the hypophyseal portal system and thereby controls the production and release of anterior pituitary hormones. The hormones released by the posterior pituitary are synthesized by the neurons of the hypothalamus. Hormones leaving the pituitary directly or indirectly influence all cells of the body. Although the hypothalamus is the main link between the nervous and endocrine systems, the cerebral
cortex exerts its influence on the hypothalamus. The cerebral cortex in turn may be influenced by external and internal stimuli. The nervous and endocrine systems are also able to coordinate the activities of the many cells of the body by acting as a communication system between the parts.

Chemically, hormones are either steroids, proteins, or modified amino acids. When released into the circulation they affect the activity of specific tissues called target tissues. The target tissues may be located close to the secreting organ, such as is the case with the hormones from the hypothalamus that act on the anterior pituitary, or the target tissues may be far away from the source of the hormone as with the anterior pituitary hormone that regulates the adrenal gland.

As our knowledge of biochemistry increases, the number of tissues and organs known to be endocrine in nature also increases. For this lab exercise we will only consider those endocrine glands which are the most prominent.

A. Pituitary Gland (Hypophysis)

This small endocrine organ (about the size of a pea in the human) is located in a depression in the sphenoid bone (the sella turcica) for protection. It is attached to the hypothalamus by the stalk or infundibulum. It consists of an anterior lobe, or adenohypophysis, and a posterior lobe, or neurohypophysis. Sometimes these lobes are simply referred to as the anterior and posterior pituitary glands. Indeed, they are 2 separate endocrine glands. The anterior pituitary develops as an outgrowth or evagination of the roof of the mouth; the posterior pituitary is an outgrowth of the brain. The infundibulum is a part of the posterior pituitary.

Using the brain models, identify the pituitary gland. Note its relationship with the hypothalamus (this relationship will be most easily observed in a midsagittal section of the brain). For guidance, see your textbook. Identify the infundibulum.

Examine a human skull and find the sella turcica. This depression in the sphenoid bone houses and protects the pituitary gland.

Examine a prepared slide of the pituitary gland. Under low power of the microscope observe the general structure of the gland (if it is not possible to see the overall structure of the gland even with low power, then use a dissecting microscope). Identify both the anterior and posterior lobes. Using high power, focus on the cells of the anterior pituitary. The cells are of three types: the acidophils, the basophils, and the chromophobes. You may be able to distinguish between the acidophils and basophils if your slide has been properly stained. Note that the cells are arranged in clusters or cords. There may be sinusoids between them containing red blood cells. The acidophil cells make and secrete the hormones prolactin and growth hormone. The other anterior pituitary hormones (thyroid stimulating
The hormone, follicle stimulating hormone, adrenocorticotropic hormone, and luteinizing hormone) are secreted from the basophils. The function of the chromophobe cells is unclear. The anterior pituitary is functionally connected to the hypothalamus by way of the hypothalamic-hypophyseal portal system. The blood vessels of this system begin in the hypothalamus and terminate in the anterior pituitary where they deliver the various releasing and inhibiting factors (hormones) from the hypothalamus.

The posterior pituitary gland is not actually an endocrine gland because it produces no hormones. The cells of the posterior pituitary are called pituicytes. Examine the posterior pituitary under the high power objective of the microscope. The nuclei of the pituicytes stain darkly. As you can see, the arrangement of the cells here is much different than in the anterior pituitary. The neurohypophysis is connected to the hypothalamus from which it originated by the infundibulum. Through the infundibulum runs a group of nerve fibers that originate in the hypothalamus and end in the posterior pituitary. It is these neurons from the hypothalamus which produce the hormones oxytocin and antidiuretic hormone. The hormones are simply released from the axon terminals within the posterior pituitary and diffuse into the blood capillaries found there.

B. Thyroid Gland

The thyroid gland consists of 2 lobes and a connecting isthmus. It is located on the trachea just below the larynx and enclosed in a connective tissue sheath. The thyroid secretes three hormones. Thyroxine (also called T4 since the molecule contains 4 iodine atoms) and triiodothyronine (or T3 because it contains 3 iodine atoms) are important for proper development and functioning of the reproductive and nervous systems and for the regulation of body metabolism. The third hormone secreted by the thyroid is calcitonin which is important in calcium homeostasis.

Identify the thyroid gland on the dissectable torso. Note its relationship to the thyroid cartilage on the larynx. The gland received its name from the thyroid cartilage with which it is associated. Also locate the thyroid gland on the human cadaver. The 2 lobes of the gland lie on either side of the anterior end of the trachea. The connecting isthmus is commonly destroyed if the trachea has been previously examined. For reference, see your textbook.

Using the microscope at low power, study a prepared slide of the thyroid gland (see your textbook). The structural and functional units of the gland are called follicles. They are rounded or irregular shaped structures composed of a single layer of cuboidal epithelium and enclosing a
glycoprotein rich in iodinated amino acids called **thyroglobulin** or **colloid**. The thyroglobulin is the storage form of the thyroid hormones T3 and T4 and is usually stained pink. It is made by the epithelial cells and secreted into the follicle. The follicle cells are then able to remove the thyroid hormones from this colloid material and release them to blood capillaries lying between the follicles upon demand from the anterior pituitary. Examine the gland with the high power objective. Note the epithelium surrounding the thyroglobulin. If the follicle is large, the epithelium may appear squamous rather than cuboidal. You may see connective tissue and blood vessels between some of the follicles. Find the **parafollicular cells**; they produce calcitonin.

C. Parathyroid Glands

There are usually four parathyroid glands situated on the posterior surface of the thyroid gland. Two are located on each lobe of the thyroid, but they are so small that you will not be able to examine them on the cadaver (see illustration in your textbook).

Examine a prepared slide of the parathyroid gland under high power magnification. There are two types of cells: principle cells and oxyphilic cells. Both secrete **parathyroid hormone** which regulates calcium and phosphorus levels in the blood. Principle cells are the most numerous but you will probably not be able to distinguish between the 2 types of cells using your slide. The glands are also well supplied by blood vessels.
D. Pancreas

This gland is both exocrine and endocrine in nature. The endocrine portion secretes the hormones **insulin** and **glucagon** which regulate carbohydrate metabolism. Both the exocrine and endocrine parts of the pancreas develop from an evagination of the gut, but only the exocrine part of the gland retains its connection (duct) to the tract.

Locate the pancreas in the cadaver. It is found in the abdominal cavity between the duodenum and the spleen. Identify it also on the dissectable torso (see your textbook).

Examine a prepared slide of the pancreas under the 10× objective. Find the **islets of Langerhans** (see your textbook). These islets compose the endocrine portion of the pancreas. The exocrine portion is composed of **acinar glands** that can also be seen. They are more numerous and smaller than the islets. Connective tissue and blood vessels may also be seen. Center one of the islets and examine it under high power. The alpha cells of the islets secrete the hormone glucagon. Beta cells produce insulin. You will probably not be able to distinguish between these two cell types on your microscope slide. You may be able to see capillaries inside the islets. It is through these blood vessels that the hormones enter the circulatory system for distribution throughout the body.

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E. Adrenal Glands

These two endocrine glands are each covered by a connective tissue **capsule** and consist of a large outer **cortex** region and a smaller inner core called the **medulla**. In the human, the adrenals are located at the superior aspect of each kidney; thus they are sometimes called the suprarenal glands. The adrenal glands produce a large number of hormones. The cortex produces steroid hormones grouped as **glucocorticoids** and **mineralocorticoids**. The various glucocorticoids are important in regulating carbohydrate metabolism whereas the mineralocorticoids are necessary for proper water and electrolyte balance. The cortex also secretes sex hormones that may serve to supplement
the supply of sex hormones from the gonads and stimulate early development of reproductive organs. The adrenal medulla secretes two neurotransmitters that are non-steroids: epinephrine and norepinephrine. These two secretions have an effect on the body similar to the effects of generalized sympathetic nervous stimulation.

In reality the cortex and medulla of each adrenal gland are two different endocrine glands. They have completely different functions, the neurotransmitters are chemically dissimilar, and they arise from different sources during embryonic development.

Locate the adrenal glands in the cadaver. They are usually superior and medial to each kidney and appear as small, hardened nodes. Find them on the dissectable torso (see your textbook).

Examine a prepared slide of the adrenal gland. Using low power, examine the entire gland. It is easy to distinguish the cortex from the medulla with this power. Now switch to high power and identify the capsule, and the first two zones of the cortex. An outer, thin zona glomerulosa, just under the capsule secretes the mineralocorticoids. The second layer, or zona fasciculata produces the glucocorticoids. The inner layer, or zona reticularis, secretes primarily androgens but small quantities of estrogen and progesterone as well. These 3 zones may grade into one another. As an aid in identification, the zona fasciculata cells appear to lie in columns between the 2 other zones. It may be difficult to determine exactly where the zona reticularis begins and the zona fasciculata ends. The center of the adrenal gland is called the medulla. It is here that the catecholamines epinephrine and norepinephrine are produced.

F. Other Endocrine Glands

Other prominent endocrine organs include the gonads (ovaries or testes). We will not examine these organs during this exercise. They are more appropriately placed in the exercise on reproduction.
G. Endocrine Physiology—Exercise 1: Glucose Tolerance Test

1. Exercise Introduction

The nervous and endocrine systems both use chemicals to communicate and to regulate physiological processes. In the traditional view, neurons mediate fast communication, either exciting or inhibiting target cells via action potentials, neurotransmitters, and synapses a scale of seconds to minutes. Endocrine glands are traditionally thought of as having slower effects on target cells, a scale of minutes to days, via chemicals (hormones) that are released into the bloodstream. Gradually, however, we are learning that the two systems continually interact and sometimes act on their target cells via similar mechanisms. Indeed, some chemicals can act as both neurotransmitter and hormone.

The hormones insulin and glucagon act in an antagonistic manner to regulate blood glucose concentration. Insulin secretion is reduced or absent in one type of diabetes mellitus. One of today’s goals is to examine the action of insulin and glucagon on a short-term basis in completely normal individuals.

2. Preparation for Exercise

NOTE: This exercise utilizes human bodily fluids that are a potential source of infection with HIV, hepatitis viruses, or other pathogenic organisms. Whenever you work with any bodily fluid (e.g. blood, urine, saliva) you should take the following precautions:

a. Handle only your own sample.

b. During each procedure, wear latex gloves. Learn how to take the gloves off without touching their outer surface with an ungloved hand. (A laboratory instructor will demonstrate this.)

c. Isolate or disinfect the sample as quickly as possible.

d. After each procedure, wash your hands with soap and disinfectant.

Waste no time in getting this experiment started, because it will require most of the session. The subjects for this test were chosen in lecture this week. To repeat,

Subjects should:

a. Be healthy

b. Not be diabetic

c. Not have a family history of diabetes
d. *Not* have blood-borne disease or have been exposed to one

e. *Not* be allergic to chocolate or nuts

f. Have no qualms about having their fingers pricked repetitively

g. Be willing (three of them) to fast several hours prior to this laboratory

Subjects 1 and 2 will consume a sugar load after fasting for at least 4 hours. Subject 3 is a fasting control and subject 4 is a non-fasting control.

Our exercise is not a true glucose tolerance test as performed in a clinic because the sugar load we use is not a measured quantity of glucose, but rather a mixture of sucrose and fructose, and is not entirely in solution. Nevertheless, we can learn from the experiment about the swings in blood glucose concentration that occur with what many consider a between-meals snack. You may be surprised by the changes in glucose concentration and by the speed with which the original concentration is restored.

The blood glucose-measuring device, the *glucometer*, is commercially available in most pharmacies. The glucometer is used by diabetic people to monitor their blood glucose level (concentration). Several times a day, diabetics measure their blood glucose level so that they can administer the proper amount of insulin to keep their blood glucose level from becoming dangerously high. Different glucometers use different methods. The glucometer system we will use is composed of a meter and reaction strips. When blood is placed on the reaction strip, glucose causes iron to lose an electron which the strip can detect. The glucometer measures the electrical potential caused by the movement of these free electrons. The amount of current in the reaction strip is directly proportional to the amount of glucose in the sample.

Record the basic information about your group’s subject and describe the sugar load. The subject should then take their first measurement of blood glucose before he or she consumes the sugar load. This measurement will be considered the fasting level of glucose. To take the measurement:

3. **Procedure**

   a. Wash hands with soap and water

   b. Make sure that the following are ready in the work area:

      i. A cotton ball

      ii. 95% ethanol

      iii. Band-Aid
iv. An autolet containing a new lancet

v. Glucometer with reaction strips

c. When the subject is ready to take a blood sample, he/she should remove a reaction strip from the vial.

d. Insert the reaction strip into the top of the glucometer until it is secure. The blood sample must be collected within 30 seconds of inserting the strip into the glucometer. If this is not possible, remove the strip and reinsert it.

e. A laboratory instructor will have shown you how to help increase blood flow to your hands. After the subject has done this little exercise and working over the blotter paper on the lab bench, be or she should uncap the lancet and use the Autolet to prick a finger. If necessary, the subject should “milk” their finger to increase blood flow.

f. The blood is applied to the reaction strip by touching the tip of the reaction strip to the drop of blood. Hold the glucometer at a 45 degree angle below the drop of blood so that blood will flow easily into the reaction strip, which acts like a straw to wick the blood inside it.

g. Once the reaction strip is filled with blood, the subject should clean their finger with alcohol using the cotton swab and then apply the bandage. The cotton swab should be disposed of immediately in the special biohazard waste bag provided.

h. In the meantime, the glucometer should have given you a blood glucose reading within 10 seconds. Record the glucose measurement and the time in the appropriate space on your data sheet.

i. The subject should remove the reaction strip from the glucometer and place it in the biohazard waste container or sharps container. The used lancet should also be disposed of in the biohazard waste container.

The subject should now consume the sugar load, within the next 10 minutes or so. The second measurement should be made 20 minutes after the fasting measurement was taken. Make measurements at 20 minute intervals for a total of 120–140 minutes.

Graph these data as the test progresses using the blank graph below. For each subject, draw a line connecting the data points and label the line. A laboratory instructor will discuss the physiology reflected by these data and you can interpret the data with the help of your textbook. What is occurring with insulin and glucagon as the lab proceeds?
4. Data—Glucose Tolerance Test

Subject #1
Sex __________________________ Wt. (kg.) ______________________
Ht. (cm.) __________________ Age ____________________________
Sugar load _______________ Time sugar load eaten ______

Subject #2
Sex __________________________ Wt. (kg.) ______________________
Ht. (cm.) __________________ Age ____________________________
Sugar load _______________ Time sugar load eaten ______

Subject #3
Sex __________________________ Wt. (kg.) ______________________
Ht. (cm.) __________________ Age ____________________________

Subject #4
Sex __________________________ Wt. (kg.) ______________________
Ht. (cm.) __________________ Age ____________________________

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<tr>
<th>Table 13-1. GLUCOSE TOLERANCE TEST</th>
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Plot the above information on this graph.
H. Endocrine Physiology—Exercise 2: Insulin Shock in a Fish

1. Exercise Introduction

This part of the laboratory will be a demonstration showing the effect of excess insulin. In diabetic people, insulin shock may occur when excessive insulin is administered inadvertently. The excess insulin results in abnormally low levels of blood glucose, or hypoglycemia, and as a result inadequate supplies of glucose are available for the central nervous system. From the course lecture or your textbook, you may have learned by now that glucose is usually the only energy source used by neurons. In addition, you know that all sensation, thought, and movement requires neuronal activity. To predict what might happen as a result of low glucose levels in neurons, consider what processes involved in neuronal activity require ATP. What might happen if your neurons could not perform these processes?

2. Preparation for Exercise

We obviously cannot inject a person with excess insulin and observe the results. Instead, we will observe the effects of insulin in a fish, which requires glucose for proper functioning of its nervous system, just as we do.

3. Procedure

A laboratory instructor will place one small fish in a beaker of water containing several hundred units of insulin. As a control, a second fish will be placed in a beaker containing only water. Over the next few minutes, observe the behavior of the two fish. What happens?

After about 15 minutes, a laboratory instructor will move the first fish to a beaker containing a 5% glucose solution. This will restore the fish's blood glucose to normal levels. Observe and describe what happens with respect to the fish's behavior. How would you verify that the effects you observed were due to the insulin and glucose?
4. Questions

a. For each of the following three situations, explain what happens to the blood glucose level during the glucose tolerance test and why these results were observed?

   i. Fasting only (control)

   ii. Non-fasting (control)

   iii. Fasting + sugar intake

b. Fish experiment: The fish became inactive when it was placed in an insulin solution and recovered when it was transferred to a glucose solution. Describe the observed behavioral changes in the fish. Explain the physiological responses of the fish to each solution in terms of the actions of the hormones insulin and glucagon.