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LABORATORY EXERCISE USING "VIRTUAL RATS" TO TEACH ENDOCRINE PHYSIOLOGY

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Animal experimentation is limited in many curricula due to the expense, lack of adequate animal facilities and equipment, and limited experience of the teachers. There are also ethical concerns dealing with the comfort and safety of the animals. To overcome these obstacles, we developed a "dry laboratory" using "virtual rats." The "virtual rat" eliminates the obstacles inherent in animal experimentation, such as inadequate budgets, as well as avoiding important animal rights issues. Furthermore, no special materials are required for the completion of this exercise. Our goal in developing this dry laboratory was to create an experience that would provide students with an appreciation for the value of laboratory data collection and analysis. Students are exposed to the challenge of animal experimentation, experimental design, data collection, and analysis and interpretation without the issues surrounding the use of live animals.


Key words: science education; "dry laboratory"; hormone; analytic thinking

The most effective means by which physiology is learned is through active participation in laboratory experimentation and analysis (2). However, experimentation is often neglected in many curricula due to the scarcity of suitable laboratory equipment, space, and experiments. The use of laboratory animals for experimentation is another obstacle because many schools do not have sufficient funding or facilities to care for live animals. In addition, some teachers may lack the experience of handling laboratory animals.

To promote laboratory experimentation, we developed a "dry laboratory" using "virtual rats." This dry laboratory is a complete experiment without additional expense, because no animals or special equipment are required for its completion. The virtual rat avoids the complications associated with the use of animals by graphically demonstrating the changes that would be observed in living animals had the experiment actually taken place. Our purpose in creating this educational tool was to develop an experience that provides an opportunity for students to explore the realm of laboratory experimentation. We have eliminated the need for special equipment or facilities. This experiment provides an opportunity for students to actively learn physiology through the collection, analysis, and interpretation of data.

The experience is designed so that students first learn the basic physiological concepts behind the endocrine system. Because of its complex and specialized nature, little experimental material for endocrine physiology exists. We have provided a less complicated means by which students are able to learn about the endocrine system. Questions within the text supplement the laboratory and promote the students' understanding of major concepts. After learning the physiological principles, the students are presented...
with an experimental design that involves the identification of an unknown hormone. The students are encouraged to complete a table that describes the effects of each hormone, thus presenting the student with a chance for small group discussion. An array of measurements are performed on the virtual rats to provide the experimental data needed. This data is presented to the students, who must use their knowledge of the endocrine system to correctly identify the unknown hormone.

Our intent for the students on completion of the experiment is to have an understanding of important physiological concepts and appreciation of laboratory data analysis. Students are able to learn more effectively if they are involved in their own education and become active, independent learners and problem solvers (3). Through this experience, we provide an opportunity for students to become active learners through small group discussion, analytic thinking, and the collection and interpretation of laboratory data. Our hope is to spark interest for future study in the field of physiology by presenting material in a thought-provoking, challenging, and inexpensive manner while encouraging the students to become independent learners.

LABORATORY EXERCISE

Objectives

A) To introduce the relationship between the hypothalamus and the pituitary gland;

B) To introduce various hormones and explain their effects;

C) To encourage small group discussion and enhance analytic thinking;

D) To have the student apply what he or she has learned to an experimental situation by identifying an unknown hormone.

Materials Needed

No materials are needed.

Time Required

Three hours is the time recommended for the completion of this exercise.

BACKGROUND AND KEY TERMS RELATED TO THIS EXERCISE

Questions are integrated throughout the text to serve as a source of discussion and aid in the understanding of key points. Questions marked with arrows (→) are used to review the previous passage, and questions marked with asterisks (**) are used to provoke thought on upcoming passages.

The organs of the body communicate with each other through the nervous and endocrine systems to coordinate their activities. The **nervous system** uses neurotransmitters and neurons to convey information to and from the brain. In contrast, the **endocrine system** uses hormones, which are chemical messengers produced by specific tissues in the body, to transmit information. These hormones travel through the bloodstream to exert their effects on distant target organs.

In a similar manner, people communicate with each other by using telephones and the postal service. The body’s nervous system is comparable to the telephone system because it sends fast, direct messages. The endocrine system is comparable to the postal service because the delivery of the message is slower. Like bulk mail, the message is more diffuse (reaches a greater area) and affects more than one person or organ. Although the hormone travels through the body via the blood, it can only affect those cells with receptors for that specific hormone. Hormones are a slower method of communication, but their effects last longer.

The command center for the endocrine system is the **hypothalamus**, a small, penny-sized portion of the brain. The hypothalamus acts as an endocrine organ that secretes oxytocin and anti-diuretic hormone (ADH, also known as vasopressin). These hormones travel down the pituitary stalk to the posterior pituitary gland where they are released directly into the bloodstream. In addition, the hypothalamus also regulates anterior pituitary gland function through the
secretion of releasing hormones: thyroid-releasing hormone (TRH), corticotropin-releasing hormone (CRH), and gonadotropin-releasing hormone (GnRH).

These releasing hormones travel through a specialized blood vessel system (known as the hypothalamic-hypophysial portal system) that connects the hypothalamus to the anterior pituitary gland. From here, they stimulate the synthesis and secretion of anterior pituitary hormones, which include thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), growth hormone (GH), adrenocorticotropin hormone (ACTH), and prolactin.

Each of these hormones is released into the bloodstream to affect specific target organs. For example, the hypothalamus secretes TRH, which travels to the pituitary gland to release TSH; TSH travels to the thyroid gland (the target organ) and stimulates the release of thyroid hormone. It is important to note that the hypothalamic releasing hormones are only required for the synthesis and release of the anterior pituitary hormones. The posterior pituitary hormones are synthesized by the hypothalamus and travel down neurons to be released from the posterior pituitary gland. Because the anterior pituitary gland secretes multiple hormones, it is frequently referred to as the "master gland." For this experiment, we will focus on the hypothalamus only as a regulator of the anterior pituitary gland. Figure 1 shows the relationship between the hypothalamus and the pituitary gland.

→ 1) Describe the relationship between the hypothalamus and the anterior pituitary gland.

→ 2) List the hormones released by the anterior pituitary gland.

→ 3) Why is the anterior pituitary called the master gland?

** 4) What is negative feedback?

The release of hormones from the endocrine system can be regulated through positive or negative feedback mechanisms. The negative feedback system can be compared with a thermostat set at a predetermined temperature (68°F). When the temperature rises above the set point (72°F), the thermostat detects the change and activates the air conditioner to cool the room. The thermostat will turn off the air conditioner once the temperature of the room drops below the set point (67°F). To keep the room at a fairly constant temperature, the thermostat assesses the situation and turns the air conditioner on or off accordingly. Figure 2 illustrates the concept of negative feedback using the above example.

In the endocrine system, negative feedback is used to inhibit further hormone secretion. When a sufficient amount of hormone has been released, it communicates or "feeds back" to suppress the releasing organ. In other words, the gland has released enough hormone to fulfill its function; this is sensed by the body, and production of the hormone ceases. Negative feedback not only inhibits the releasing organ, but can also inhibit the pituitary gland and/or hypothalamus. By using a negative feedback system, the body produces only the amount of hormone it needs without wasting its resources. Conversely, in positive feedback, the end product further stimulates the releasing organ. This form of feedback is less common.

The pathways of three hormones are examined in this experiment: thyroid hormone, cortisol, and testosterone. The hormonal pathways are similar in all three cases. It is important to realize that the hypothalamus secretes a releasing hormone to regulate each of the hormones secreted from the anterior pituitary gland. In this way, the hypothalamus is like a command center. If the hypothalamus is not stimulated, the hypothalamic releasing hormones (TRH, CRH, and GnRH) will not stimulate the anterior pituitary gland to secrete its hormones.

The hypothalamus releases TRH, which travels to the anterior pituitary gland via the bloodstream to stimulate production of TSH. TSH travels to the thyroid gland (located by the trachea) to stimulate the production and release of thyroid hormone. Thyroid hormone influences the growth rate of many body tissues and is necessary for proper central nervous system development. Its main function is to increase a person’s basal metabolic rate (BMR) and to increase heat production. An excess of thyroid hormone can negatively feed back to inhibit further thyroid hormone release from the thyroid gland, TSH secretion from the
anterior pituitary gland, and/or TRH release from the hypothalamus.

Similarly, ACTH is released from the anterior pituitary gland in response to CRH secreted from the hypothalamus. ACTH stimulates the adrenal glands (located on top of the kidneys) to secrete cortisol, which promotes the breakdown of proteins and fats and helps the body adapt to stress. Cortisol functions to provide the body with fuel by breaking down (catabolism) the materials of the body. Under normal conditions, excess cortisol in the bloodstream will negatively feed back to the hypothalamus (to inhibit CRH release), anterior pituitary gland (to inhibit ACTH secretion), and/or to the adrenal gland (to inhibit further cortisol release). The release of CRH is regulated by negative feedback, circadian rhythms, and stress. Cortisol can also act as an immunosuppressive and anti-inflamma-
tory agent. If cortisol is administered in large doses, its immunosuppressive properties will cause the organs of the immune system to shrink. In this experiment, the thymus gland will represent the organs of the immune system.

- 5) Describe the effects of thyroid hormone.
- 6) Describe the effects of cortisol.

** 7) Describe the role of LH in both males and females.

LH is released from the anterior pituitary gland in response to GnRH secreted from the hypothalamus. LH is seen in both males and females but has different functions. In the male, LH travels to the Leydig cells that are located in the connective tissue between the seminiferous tubules of the testes. The Leydig cells release testosterone, which is responsible for the male sex drive and secondary sex characteristics, such as increased body hair and a deeper voice. An excess of testosterone can cause an increase (anabolic) in muscle mass. Negative effects of testosterone are male pattern baldness and increased secretion of the sebaceous glands, which can lead to acne. Figure 3 presents the relative anatomy of the male reproductive tract.

In the female, LH causes the follicle (developing egg) in the ovary to secrete estrogen. Estrogen participates in either a positive or negative feedback loop, depending on the stage of the menstrual cycle. In the preovulatory and postovulatory phases, estrogen regulates the release of LH through negative feedback. However, there is a large rise in levels of LH just before ovulation (release of the egg from the ovary) due to a positive feedback mechanism. During this interval, the secretion of estrogen from the follicle further stimulates the release of LH from the anterior pituitary gland. The increased levels of LH are essential for ovulation to occur. Estrogen causes the development of female secondary sex characteristics and

FIG. 2. Negative feedback compared with a thermostat controlling room temperature. Solid lines and (+) represent an enhancement of activity, whereas dotted lines and (−) represent an inhibition of activity.

FIG. 3. Organs of the male reproductive tract.
sustains the female reproductive tract. A woman who lacks ovaries (and therefore follicles) will not produce estrogen. However, the pituitary gland will secrete excess LH because the feedback inhibition no longer exists. Excess levels of estrogen cause early sexual development in the female as do high levels of testosterone in males.

To simplify the relationship between the reproductive and endocrine systems, we will concentrate only on the male system. The female reproductive system is more difficult to study than the male reproductive system because it is continuously cycling. The pathways of all three hormones can be understood by looking at a visual representation in Fig. 4 (Fig. 4 also demonstrates the pathways of the hormones that will be used throughout the experiment, thus serving as an aid in the analysis of laboratory data).

The glands and tissues of our body enlarge (increase in size) if they are continuously activated; this is called hypertrophy. For example, a person who lifts weights will continually stimulate the activated muscles, resulting in hypertrophy. This can be easily observed when comparing a bodybuilder to an average person; the bodybuilder’s muscles appear larger in comparison. In contrast, if a gland or tissue is continuously inhibited it will shrink in size or atrophy. For example, if a cast is placed on a person’s arm for 6 wk and then removed, a drastic reduction in muscle mass can be seen. The cast prevented any movement (stimulation) of the limb, allowing atrophy to occur.

→ 8) Explain the positive feedback loop observed in LH regulation.

→ 9) Describe the difference between hypertrophy and atrophy.

** 10) Consider the differences between hyperthyroidism and hypothyroidism. What are some characteristics of each?

** 11) What are the effects of decreasing testosterone?
There are many diseases that may result from a deficiency or excess of hormones. These hormonal imbalances may lead to changes in organ or gland size (hypertrophy or atrophy). **Hyperthyroidism** is the excessive production of thyroid hormone. The most common cause of hyperthyroidism is Grave’s disease; the symptoms include increased BMR, a constant feeling of warmth, nervousness, and an enlarged thyroid gland (known as goiter). In contrast, **hypothyroidism** is the result of decreased levels of thyroid hormone. A patient with hypothyroidism will present symptoms of low BMR, a decreased appetite, abnormal central nervous system development, and an intolerance to cold.

**Cushing’s syndrome** is the result of excess secretion of cortisol (hypercortisolism). The symptoms of Cushing’s syndrome include personality changes, hypertension (high blood pressure), osteoporosis (weakening of bones due to loss of calcium), and weight loss. If an excess level of cortisol remains in the body, protein degradation will occur leading to a “wasting” effect. Hyposecretion (decreased secretion) of cortisol is characterized by symptoms such as defective metabolism, mental confusion, and a decreased ability to adapt to stress.

Decreased amounts of testosterone in the body primarily affect the sexual organs. If testosterone levels are low, males will not develop normally and will have sperm counts too low to fertilize an egg. The condition of excess levels of testosterone is rare but causes premature sexual development.

Figure 5 presents a concept map that students may find useful in organizing the basic principles of endocrine physiology.

**LABORATORY PROCEDURE**

This exercise is designed to determine the identity of an unknown hormone by observing the effect it (the hormone) had on the organs of the male rat. The exercise is a modification of an experiment from a laboratory manual that is currently in press (1).

The following format presents two different options for the teacher. These options are only suggestions,
and the teacher may develop other ideas for presenting the material. The number of students per group and the time allotted may differ from class to class.

**Option 1**
The class should be divided into small groups of three or four students per group. Each student in the group should receive a copy of Table 1. Using the background material, the students should complete Table 1 before class. The teacher will present each group with an unknown hormone and the corresponding autopsy data. The students will use the flowchart (Fig. 4), Table 1, and the autopsy data to determine the unknown hormone. At the end of class, the students will present their solution and rationale for their identification of the unknown hormone. If time permits, the students are encouraged to determine the identity of the remaining unknowns and provide a solution and rationale for each hormone.

**Option 2**
The class should be divided into small groups of three or four students per group. Each student should receive a copy of Table 1 and Figs. 7–13. Using the background material, the students should complete Table 1 before class. The students will determine all six unknowns and write a laboratory report discussing the solution and rationale for the identity of each hormone. The laboratory report may be written in class or as homework.

The data for this laboratory were compiled from seven sets of male laboratory rats, two rats per set; one set was the control group and the remaining six were experimental groups. The rats were all male to simplify the study of the relationship between the reproductive and endocrine systems. In each set of rats there was an “intact” rat and a “castrate” rat. The castration involved removal of the testes to eliminate testosterone production. The two rats (normal and castrate) of each group were treated alike in all other ways (food, water, etc.). All rats, except for those in the control group were injected with a hormone on a daily basis for 2 wk. Autopsies were performed on the animals at that time.

The group of students performing this exercise were very disorganized and rushed through the work, making errors in labeling the bottles of hormone. The students obtained the following results for organ weights after the autopsies were performed. In this short period of time, the students noted amazing changes in the size of certain organs when they compared the experimental group of rats with the control group. Using the flowchart (Fig. 4), Table 1, and the autopsy data, match the unknown rat groups with their respective hormones. The bottles on the

<p>| TABLE 1 |</p>
<table>
<thead>
<tr>
<th>Comparison of hormonal effects on different organs (to be completed by students)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRH</td>
</tr>
<tr>
<td>Intact</td>
</tr>
<tr>
<td>Pituitary gland</td>
</tr>
<tr>
<td>Thyroid gland</td>
</tr>
<tr>
<td>Adrenal glands</td>
</tr>
<tr>
<td>Thymus gland</td>
</tr>
<tr>
<td>Testes</td>
</tr>
<tr>
<td>Prostate</td>
</tr>
<tr>
<td>Seminal vesicles</td>
</tr>
<tr>
<td>Body weight</td>
</tr>
</tbody>
</table>

A + denotes an increase in size. A − denotes a decrease in size. Place the letters NC in the box where no change occurs. TRH, thyroid-releasing hormone; TSH, thyroid-stimulating hormone; ACTH, adrenocorticotropic hormone; LH, luteinizing hormone.
refrigerator shelf were ACTH, cortisol, LH, TSH, TRH, and testosterone.

To help in determining the identity of the unknown hormones, the student should look for changes between the control values and the values of the unknown hormone (both the intact and castrate animal). The changes between the control rats and the rats that were treated with the unknown hormone should be >20% if they are to be considered significantly different. If the change is <20%, it is attributed to experimental or biological error. Experimental errors may include small errors in calibration procedures, measurements, or instrumentation. Any variability that occurs because of the differences between animals is considered biological error.

Figure 6 represents the organs of the rats used in the experiment.

Figure 7 shows your set of control rats; the data are the results of the autopsy.

Determine the identity of hormone 1 using the data from the autopsy listed in Fig. 8, Table 1, and Fig. 4.

Determine the identity of hormone 2 using the data from the autopsy listed in Fig. 9, Table 1, and Fig. 4.

Determine the identity of hormone 3 using the data from the autopsy listed in Fig. 10, Table 1, and Fig. 4.

Determine the identity of hormone 4 using the data from the autopsy listed in Fig. 11, Table 1, and Fig. 4.

Determine the identity of hormone 5 using the data from the autopsy listed in Fig. 12, Table 1, and Fig. 4.

Determine the identity of hormone 6 using the data from the autopsy listed in Fig. 13, Table 1, and Fig. 4.

**ANALYSIS**

1) What was hormone 1? Explain your answer.

2) What was hormone 2? Explain your answer.

3) What was hormone 3? Explain your answer.

4) What was hormone 4? Explain your answer.
5) What was hormone 5? Explain your answer.

6) What was hormone 6? Explain your answer.

TEACHER’S COPY OF ANSWERS TO TEXT QUESTIONS

1) The hypothalamus is an important control center for regulating the release of hormones from the anterior pituitary gland. Releasing hormones, such as TRH, are released into the blood and travel through a specialized blood vessel to the anterior pituitary gland. There, TSH is secreted from the anterior pituitary gland and enters the blood system.

2) Hormones released by the anterior pituitary gland are TSH, LH, FSH, GH, ACTH, and prolactin.
3) The anterior pituitary gland is known as the master gland because it secretes multiple hormones.

4) Negative feedback is a regulatory mechanism used by the endocrine system. Once a sufficient amount of hormone has been released, it will feed back to inhibit the organ from further releasing the hormone. The hormone may feed back to the releasing organ, pituitary gland, and/or the hypothalamus. For example, TSH feeds back to the hypothalamus and anterior pituitary gland to decrease TRH and TSH production, respectively.

5) Thyroid hormone:

- Regulated by TRH from the hypothalamus, which causes the release of TSH from the anterior pituitary gland.
Regulates TRH and TSH release through negative feedback inhibition.

Influences the growth rate of tissues and is essential for the proper development of the central nervous system.

Functions to increase a person’s BMR and increase heat production.

6) Cortisol:

- Regulated by CRH from the hypothalamus, which causes the release of ACTH from the anterior pituitary gland.

- Regulates CRH and ACTH release through a negative feedback inhibition.
- Stimulates the breakdown of proteins and fats and helps the body adapt to stress.

- Can function as an anti-inflammatory drug and an immunosuppressive (therefore, when in excess, cortisol will cause a decrease in immune system function).

7) The role of LH is different in males and females. In males, LH travels to the Leydig cells to allow the release of testosterone. Testosterone is responsible for the males’ secondary sex characteristics. In females, LH causes the follicle in the ovary to secrete estrogen. Estrogen is responsible for the females’ secondary sex characteristics.

8) LH positive feedback, seen in the females, causes the follicle to release estrogen. Estrogen feeds back to the anterior pituitary gland to cause an increase in LH.
secretion, which results in a surge of LH just before ovulation. The increased levels of LH are essential for ovulation.

9) Hypertrophy is an increase in organ size due to a continuous stimulation or lack of inhibition by the hormones. Conversely, atrophy is a decrease in organ size due to either the continuous inhibition or lack of stimulation by hormones.

10) Hyperthyroidism is an increase in the production of thyroid hormone resulting in an increased BMR and heat production. The most common cause of hyperthyroidism is Grave’s disease. Hypothyroidism is a decrease in the production of thyroid hormone, thus decreasing the BMR, heat production, and appetite.

11) A decrease in testosterone will primarily affect the male sex organs. If the levels of testosterone are low,
males will not develop normally and will have a sperm count too low to fertilize an egg.

**TEACHER’S COPY OF LABORATORY ANSWERS**

Table 2 shows the completed Table 1.

**Hormone 1: ACTH**

ACTH stimulates the release of cortisol from the adrenal glands; however, cortisol is only released in the maximum amount the body can produce (physiological dose). The increase of cortisol causes the negative feedback mechanism to inhibit the production of ACTH from the anterior pituitary gland, which causes a decrease in gland size. The adrenal glands increased in weight because they were being stimulated, whereas the thymus gland had a reduction in weight because of cortisol’s immunosuppressive action. The thymus is the site of lymphocyte processing and development. Cortisol also caused a general

**FIG. 13.**

Autopsy results from rats treated with hormone 6.
decrease in body weight because it promotes protein degradation and lipolysis. ACTH does not affect the reproductive organs.

Hormone 2: LH

LH affects the reproductive system. There was a size increase in the testes, prostate, and seminal vesicles in the intact rat due to the release of excess testosterone. An increase in body weight is also seen because of the physiological effects of testosterone. In the castrate rat, there were no testes for LH to stimulate nor testosterone to be released; therefore, the size of the prostate and seminal vesicles did not change in the castrate rat. The size of the pituitary gland in the intact rat decreased in size because of the negative feedback effects of LH.

Hormone 3: Testosterone

When testosterone was added from an external source, the castrate and intact animals appeared the same. The large increase in body weight was due to the pharmacological doses of the androgen, causing an increase in muscle mass (note that men are generally bulkier than females). There was a decrease in the testes mass because testosterone negatively feeds back to inhibit its own release from the testes. Similarly, the pituitary gland also decreased in size because of the negative feedback from the testosterone. An increase in the mass of the seminal vesicles and the prostate was seen because testosterone functions to maintain the male reproductive system.

Hormone 4: TRH

The most obvious difference was the increase in pituitary gland size due to excess stimulation by TRH. The thyroid increased in size because TRH stimulated the release of TSH. There was a general wasting effect on all body organs, because hyperthyroidism causes an increase in metabolism, thus the decrease in body weight and organ size. TSH does not significantly affect the reproductive organs.

Hormone 5: Cortisol

Cortisol causes protein degradation and lipolysis (breakdown of fats) and, therefore, a general decrease in body mass. The pituitary and adrenal glands decrease in size because they are inhibited by the negative feedback of cortisol. The pharmacological dose of cortisol caused a large reduction of weight in the thymus gland because of its immunosuppressive effects.
action. Cortisol does not significantly affect the reproductive organs.

Hormone 6: TSH

The size of the pituitary decreased because TSH negatively feeds back to the pituitary gland and the hypothalamus. Only the thyroid glands increase in size with excess stimulation by TSH. The rest of the body underwent a wasting effect due to the release of excess TH, which raised the BMR, causing the body weight of the rat to decrease. TSH does not affect the reproductive organs.

SUMMARY

Laboratory experimentation is often neglected in many curricula. By creating a dry laboratory using virtual rats, we have eliminated many obstacles associated with laboratory experimentation. Our goal in creating this laboratory was to provide the students with an opportunity to actively learn physiology through the collection of data, analytic thinking, and small group discussion.

The exercise begins with background information on basic endocrine physiology. Thought-provoking questions were presented throughout the text as a focus for small group discussion. The laboratory presents data obtained from the autopsies of seven sets of virtual rats that were given unknown hormones. The students must correctly identify the unknown hormones based on the previous background material, small group discussion, and analytic thinking.

On completion of this exercise, it is our intent that students not only have a basic understanding of endocrine physiology, but also are encouraged to continue their exploration of science. The exercise provides a simple yet challenging encounter with endocrine physiology. The virtual rat provided the student with an opportunity to dive into the realm of animal research without its many complications. Students not only learn the principles of endocrine physiology, but also learn to appreciate laboratory work and analysis. Our goal in creating this exercise is to introduce students to the joys and mysteries of physiology as well as to generate interest for future scientific study.

Christopher T. Hsu, Sandhia Varyani, and Eilynn Sipe were supported by the Summer Fellowship Program at Northeastern Ohio Universities College of Medicine. J. Paul Layshock was supported by the American Physiological Society’s Frontiers In Physiology Science Research Program for Teachers.

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Received 24 June 1996; accepted in final form 1 September 1997.

Suggested Readings


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