Supplemental Material

CBE—Life Sciences Education

Godin *et al.*

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What is motivation?

- Based on an individual's beliefs about:
  1) Can I do this?
     - **Perceived competence** (self-efficacy)

  2) Why do I want to do this?
     - **Intrinsic**: engage for enjoyment, task is valuable/interesting, to learn or improve
     - **Extrinsic**: engage to get a reward, avoid punishment, impress others, outperform others

How is the summer program designed to support motivation?

- Real-world, challenging tasks
- Self-generated academic work
- Active involvement in learning
- Supports feelings of belonging
- Informal, effort-based evaluation

Supporting Perceived Competence

GOAL: Want students to feel like they can learn science (biology, chemistry, pharmacology)

TIPS :

- Hold high but realistic expectations (remember these are undergrads with limited science background)
- Support students to successfully solve challenging problems/answer questions on their own
- If students are not successful, help them to see other approaches that might lead to success in the future

Supporting Intrinsic Motivation

GOAL: Want students to enjoy science, focus on learning and understanding

TIPS: Support autonomy

- Provide explanations, rationales
- Allow students to make choices
- Be patient, give students time to work through activities on their own

TIPS: Make learning meaningful

- Make connections between course materials and real life
- Actively involve students in learning

Supporting Intrinsic Motivation

GOAL: Want students to enjoy science, focus on learning and understanding

TIPS: Support Feelings of Belonging

- Help students relate to you
- Foster positive relationships among LEAP students
- Encourage atmosphere of respect and warmth

TIPS: Focus Evaluation on Learning, Improvement

- Provide private feedback focused on the process (not the product)
- View mistakes an opportunity for learning

Reducing Extrinsic Motivation

GOAL: Do NOT want students to focus on outperforming others, getting good grades, demonstrating competence

TIPS: Reduce competition

- AVOID directly comparing students or pointing out particular students as smart
- AVOID activities that support competition or social comparison among students

TIPS: Reduce controlling behaviors

- AVOID being overly directive (instead support autonomy)
- Do NOT use rewards, prizes, etc.

Bar Flies: Pharmacogenetics of Ethanol Metabolism

Objectives:

1) Describe the metabolism of alcohol.
2) Describe how a gene polymorphism in ADH can increase the risk of alcohol addiction
3) Explain how a gene mutation can affect protein levels vs protein structure
4) Discuss how to convert observation data (qualitative) into quantitative data for analysis
5) Explain the basic use of an ANOVA for statistical analysis

Background:

Several types of gene variations can influence alcohol intoxication (rate and extent) in people, leading to different risks of the development of alcoholism. Such gene variations can be studied in animal or organism models of disease. The *Drosophila melanogaster* is an excellent model for studying the behavioral effects of alcohol, based on the work of Ulrike Heberlein* at the University of California at San Francisco. Like people, the flies possess the alcohol dehydrogenase (ADH) gene, which controls the production of the major alcohol oxidizing enzyme, ADH. Flies are attracted to alcohol to get their food, but at higher concentrations of alcohol they become intoxicated and can even die of alcohol poisoning. Thus, having a functional ADH enzyme serves as a survival mechanism.

Behavioral activity during intoxication (summarized by Ogueta eta l., 2010):
In fruit flies, intoxication causes initial hyperactivity followed by the flies becoming increasingly uncoordinated and sedated. As brain and hemolymph ethanol concentrations increase, the flies lose their ability to control posture when challenged, and after long exposure lose control over walking and flying movements. Inebriated adult flies also show an increase of courtship activity.

There are several isoforms of the human ADH gene that affect the rate and extent of alcohol oxidation. The same is true in Drosophila. Some have an allele mutation in their ADH gene leading to flies with null or greatly reduced ADH activity ("ADH minus" flies). These flies become easily intoxicated by alcohol (like humans!) and can ultimately die of alcohol poisoning.

Flies possessing a functional ADH gene have the ability to oxidize ethanol to acetaldehyde:

$$NAD^+ + ADH + alcohol \text{ --------> } NADH + acetaldehyde + H^+$$

In this activity, students will develop a laboratory experiment comparing the rate and extent of alcohol-induced intoxication in normal ("wild-type") flies and ADH- flies with the ADH gene mutation. Students will rate the fly behavior (blind to the treatment condition) at different times after coming up with a scoring scale for intoxication. After deciding on a specific time-point to compare the effects of alcohol on both types of flies, they will use a statistical analysis (ANOVA) to determine significant differences for main effects of alcohol treatment and polymorphism and interactions at specific doses.

Note to instructors: Before starting the activity review the basics of alcohol oxidation.

$$\text{Alcohol} + NAD^+ \xrightarrow{\text{ADH}} \text{acetaldehyde} + NADH + H^+$$

$$\text{acetaldehyde} + NAD^+ \xrightarrow{\text{AldDH}} \text{acetic acid} + NADH + H^+$$

1. Why are enzymes so important to make biological reactions go?
2. What other kinds of diseases (other than alcoholism) are the result of enzyme deficiencies in the body?

Materials:

Fruit flies (*Drosophila) ADH- and ADH+* (40 of each per student group)
Fly vials and sponges (8 per group)
Alcohol - White wine or 95% ethanol
cotton balls (32 per group)
stirring rods (8 per group)
pipettes
small (10 ml) graduated cylinders
delicate paint brushes
ice bucket

Prior to the experiment, tell students that fruit flies are attracted to alcohol, which is normally made in food that is decaying. If the alcohol is high enough concentration, the flies can get intoxicated.

Discuss with them that fruit flies are a great genetic model for many disorders in humans.
Ask students: "How would you test for alcohol intoxication in fruit flies that have polymorphisms in an enzyme that oxidizes alcohol? What would happen to flies that can't oxidize alcohol?"

Guide the students to design a procedure to answer these questions. Things they will need to find out are:

- The enzyme that oxidizes alcohol
- What kind of polymorphisms exist for this enzyme (ADH) in flies
- How the oxidation of alcohol by ADH proceeds
- Why would a fly have ADH?
- What is a toxic dose of alcohol for a fly?

Students should come up with a control condition (water) and 3 doses of alcohol (white wine is 13%, but can make a higher concentration by adding 95% ethanol). Guide them to come up with a list of conditions similar to the one shown below.

ADH- flies, water	ADH+ flies, water
ADH- flies, alcohol-dose 1	ADH+ flies, alcohol-dose 1
ADH- flies, alcohol-dose 2	ADH+ flies, alcohol-dose 2
ADH- flies, alcohol-dose 3	ADH+ flies, alcohol-dose 3

Then, students should discuss how they will observe and record their data. They should construct a behavioral rating scale for their observations. Here's an example (the whole class should use the same scale).

Behavior score	Behavior
1	Flying around
2	Some movement
3	Little movement
4	No movement but alive
5	Dead

Next, they should make a class decision on a proper data table for recording all their data for their 5 flies at specific time points over 24 hours. Instructor should construct the table on the computer (with projection) and when it is finalized, the instructor should print out enough copies for each student.

A table could look something like this:

Condition Code:_____

Time after exposure	Behavior Score				
	"1"	"2"	"3"	"4"	"5"
15 min					
30 min					
60 min					
2 hours					
4 hours					
8 hours					
24 hours					

Procedure:

Instructors:

For each lab room: Presort flies into an ADH+ vial and an ADH- vial. These flies can be anesthetized when class starts by chilling them on ice for 5-10 minutes. Keeping them on ice afterwards also slows their revival.

1. Give each group 8 empty vials, about 32 cotton balls and 8 stirring rods.

2. Using the stir rod, wedge 3-4 cotton balls in the bottom of each vial.

3. Each student chooses one of the experimental conditions to set up and labels the tube with a piece of tape with their name and the condition (e.g., ADH-, water)
   (Note: later on the instructors will remove the tape and code the vials (A, B, C, etc.) so that the students are "blind" to the treatment condition)

4. Students add 5 mls of either water or varying concentrations of alcohol (white wine or 95% ethanol) to their vials. Each cotton ball should be soaked but not submerged. Using the stirring rod, tap down the cotton balls to wedge them in place and then drain off excess fluid into a beaker or paper towels. They can test the cotton ball's security by inverting the vial.

   Note: Make sure the cotton ball is well-drained, so as not to drown the flies. The cotton ball should be very damp, but not leak liquid when pressed.

5. Dry the inside walls of the vials if they are wet. (Flies can drown in drops of fluid)

6. Give your group the 2 vials of ADH +/- anesthetized flies. The flies in the vial should not be moving. If the flies wings or legs appear to trembling they should be put on ice longer. As soon as the flies warm up, they will wake up and fly away.

7. Open the chilled vial and pour the flies onto a piece of paper. Using the paintbrush, gently sweep 5 flies of one strain to put into each of the labeled vials. Stopper the vial, but leave the vial on its side so the anesthetized flies will not drown in the wet cotton.

8. When the flies revive, turn the vial upright. If less than 3 flies recover, obtain more to make 5 total.

9. The instructor should replace the tape on each vial with a new piece of tape with a code: A, B, C, D, etc. Store the code for the original labels in a notebook. Students should discuss the advantage of being "blind" to the treatment condition (i.e., to eliminate bias when gathering observational data).

10. Return vials to the students – make sure that they don't know which condition they will be observing.

11. Students start their observations and record their behavior over the next 24-hours at the time points that they decided to use.

12. Students should share their data with the other members of their lab and decide which time point they want to use for the data analysis. This should be a time point at which they have a good indication of intoxication. They should also determine what score they will use as having reached "intoxication" (e.g., score of at least 3). They should email you the time point and score that they have chosen as a group that will be used for the data analysis.

13. During the next class, compile the data to show as a table or plot. Then perform the appropriate statistics.

    One way to turn qualitative data into quantitative data is to plot the individual scores as a scatterplot at the agreed-upon time point on a graph with all the doses and both ADH polymorphisms. Put a circle or an X for each fly in each condition. They can draw a line for the median. Note: some believe using a mean for scored data as an average is not appropriate, but this opinion varies widely.

    The data can be analyzed with an ANOVA and a post-hoc test such as Sheffe's test to compare the behaviors of the ADH + vs ADH - flies. (Ideally, non-parametric statistics would be best for scaled data, but this is beyond our discussion today).

14. Have students run the ANOVA and Sheffe's test using the Stats program of choice (Prism).

15. Have a discussion on data analysis, to compare data within and between the different groups. Students need to be sure to differentiate fly death due to alcohol poisoning versus death due to other factors (e.g., poor handling, etc.)

16. Ask students to extrapolate their results to humans; people with highly functioning ADH metabolize alcohol well and have less intoxication, while people with the gene that makes a poorly functioning ADH (or no ADH) are likely to become very intoxicated since they can't get metabolize alcohol very quickly.

By Karen Hopkin

PROFILE

Drunken *Drosophila*

Ulrike Heberlein started out studying fruit fly eyes. So how did she end up inventing the inebriometer?

It began with a simple observation in 1993. Ulrike Heberlein – then an investigator at the Ernest Gallo Clinic and Research Center at the University of California, San Francisco – placed a fruit fly in a little chamber, gave it a puff of alcohol vapor, and monitored its reaction. "What we observed is that a fly behaves just like any other organism when under the influence of alcohol," she says. "First thing it does is become really excited. It runs around really quickly and starts bumping into things." Keep the alcohol coming, and inebriated flies grow increasingly uncoordinated. "Eventually they just sort of fall over and lie there," says Heberlein. Recovery from a binge is no prettier. "They get up, they fall down again. You just observe these tiny little flies and you can relate to them," she says. "You think, this is awfully similar to something that maybe I experienced once in my life."

In the years that followed, Heberlein, now a professor at UCSF, turned those sympathetic observations into a career of studying the effects of drugs and alcohol on fruit flies. She and her colleagues have discovered a handful of genes that influence how these creatures respond to ethanol and cocaine, and they hope that their findings will lead to a better understanding of intoxication and addiction in more complex organisms, including humans. "I think she's more motivated than a lot of basic scientists by a desire to do something medically significant," says Cori Bargmann, a Howard Hughes Medical Institute (HHMI) investigator at the Rockefeller University. "Her project is based on a real conviction that alcohol and drug abuse is a human tragedy and [that] people should do something about it."

From the Zambezi to Berkeley

Heberlein didn't set out to devote her life to drunken *Drosophila*, or even to science. Although she studied biochemistry as an undergraduate in Chile – an experience she imagined would teach her "how life works at a molecular level" – Heberlein was leaning toward pursuing a life of ▸

outdoor adventure. "I did river rafting and mountain climbing for a couple of years in the early 80s," she says. But just as she was about to pack her bags for Zimbabwe to help her boyfriend run a rafting operation on the Zambezi River, Heberlein says, "I realized that maybe going back to science wasn't a bad idea. At least in science I knew what I was doing."

That realization brought Heberlein to Berkeley in 1983, to the laboratory of HHMI investigator Robert Tjian. For her thesis project, Heberlein was searching for the transcription factors that bind to the promoter of one of the few fly genes that investigators had identified: alcohol dehydrogenase. Along the way, she worked out the first cell-free transcription system using fruit fly embryos at different stages of development. "Believe it or not, the *Drosophila* field was well covered genetically, but virtually nobody did biochemistry," says Tjian. "Ulrike really cracked open the entire transcription system in *Drosophila*, which has been paying dividends ever since."

The time in Tjian's lab gave Heberlein "a very solid education in biochemistry," she says. But it also awakened in her a longing to manipulate genes in an animal. Working with isolated bits of DNA and purified proteins in a test tube, she says, "I felt like I had a little too much control over what was happening. And I thought that if I worked with a whole organism, and used genetics rather than pipetting to change conditions, that ultimately I would learn something about what's important to the organism."

In 1988, this desire to learn a genetic approach drove Heberlein across the hall for a postdoc in Gerry Rubin's lab, where she began to study the development of the fly eye. "It's this incredibly precise, beautifully structured compound eye, with 800 units that are aligned almost like a crystal," she says. Heberlein learned the techniques that would allow her to probe the genes that initiate the wave of differentiation that sweeps across the developing eye. She was again breaking new ground experimentally and biologically.

"Ulrike has a history of initiating interesting fields," says Jay Hirsh of the University of Virginia in Charlottesville. "I think she was one of the first to get into the whole question of what causes movement of the morphogenetic furrow during eye development in the fly," he says. "This has become a huge field unto itself."

Like Flies to ... Alcohol

After her postdoc, Heberlein needed to find a job. She learned of an opening at the Gallo center, which had been established to study the effects of alcohol and drugs of abuse on the brain. Heberlein wrote to then-director Ivan Diamond and described her interest in the position. "I said I had studied alcohol dehydrogenase as a graduate student, which made me sound like I knew something about the field, although I really didn't at all," she recalls. Diamond was receptive, but asked Heberlein to outline something more specific. "So I started reading and thinking about how you can measure behaviors induced by alcohol in flies," says Heberlein. "I wrote a two-page proposal. Next thing, I had a job interview, and then I had a job."

> "After the cheapdate paper, flies and alcohol were no longer a laughing matter."
> —*Adrian Rothenfluh*

Launching her career in 1993 at the Gallo, her friends and colleagues say, was a curse and a blessing. "It wasn't exactly the easiest place for a young researcher to get going," says Tjian. The Gallo is physically isolated, located across the Bay Bridge in Emeryville, rather than on the UCSF campus, which he says made it more challenging for Heberlein to recruit talented graduate students and postdocs to aid in her pioneering work.

On the other hand, says Bargmann, the Gallo was willing to support studies that weren't ready for prime NIH funding. "Ulrike's work got started at a point where the whole thing just seemed too wacky for words," she says. "But it's those early times when people think you're crazy that somebody has to step up and support you." The Gallos, who'd donated a portion of their winery profits to address some of the problems associated with alcohol abuse, were willing to give Heberlein that support.

Funding in hand, Heberlein needed to develop a screen that would allow her to tease out the genes that control how a fly responds to alcohol. Enter the inebriometer. The device is a tall cylindrical column, lined with slanted platforms, through which alcohol vapors can be circulated. Flies are placed at the top of the apparatus and, as they become inebriated, lose their footing and tumble to the bottom. Flies fall faster the more sensitive they are to the effects of alcohol. Using the inebriometer, which she built from scratch, Heberlein isolated mutants that are either more sensitive or less sensitive to intoxication than are wild type flies.

"I remember how the audience chuckled, half amused, half bemused," when Heberlein discussed her inebriation assay at a fly meeting in 1998, says postdoc Adrian Rothenfluh. That changed the following year, though, when Heberlein and her team isolated *cheapdate*, a mutation that lowered flies' resistance to the intoxicating effects of alcohol. "After the cheapdate paper, flies and alcohol were no longer a laughing matter," says Rothenfluh.

Cheapdate, cAMP, and Memory

Cheapdate disrupts a peptide called amnesiac, which Chip Quinn's lab at Massachusetts Institute of Technology had previously identified. Amnesiac is involved in learning and memory, reinforcing the theory (proposed by others in the field) that addiction might be a maladaptive form of learning. Heberlein and her colleagues are still trying to identify the receptor to which the neuropeptide binds. They have learned that cheapdate activates cAMP signal transduction in a subset of fly neurons; the same cAMP pathway has been implicated in mediating alcohol's effects in mammals. "So we think we're barking up the right tree," says Heberlein.

The approach was a gamble, says UCSF colleague Cynthia Kenyon. "Going in, it wasn't clear whether she could find single genes that would produce specific effects instead of just a jumble. Or maybe there would have been nothing there to study," she says. Heberlein's experimental rigor

allowed her to find specific genes and to follow through and determine their functions, says Tijan. "Cloning a gene might be easy. But you then have to go and actually figure out what the heck it does."

Although the connection with learning was a surprise, the discovery highlights "why flies are great for this kind of study," says Linus Tsai, Heberlein's former MD-PhD student. "With flies you can do an unbiased search for genes and molecules that might be involved in drug response. You're not limited to what's already known."

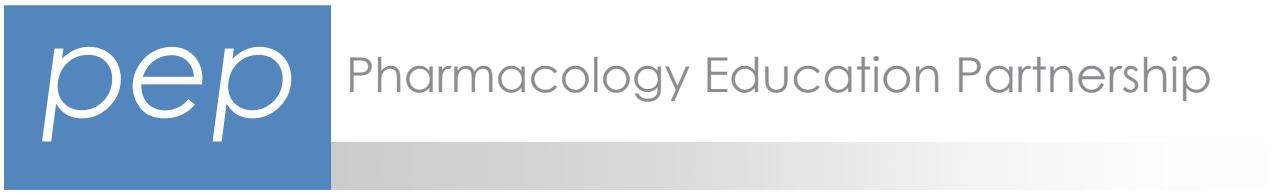
Heberlein and her colleagues continue to push those limits, expanding their studies to include cocaine, nicotine, and other drugs of abuse. "We throw everything we can get our hands on at these flies," she says, and additional genes have emerged. For example, Heberlein, Tsai, and other lab members have conducted cocaine sensitivity studies, which have turned up lmo, a tiny little protein expressed in a set of circadian pacemaker cells. These neurons help coordinate various rhythmic behaviors, including locomotion, an activity that goes awry when flies are exposed to cocaine.

Alongside the fly studies, Heberlein is working on proving that the corresponding genes play a role in drug response in mice. "It was a bit of an extrapolation to believe that flies would be a good model to study addiction," says Heberlein. "A lot of people questioned whether that was the right way to go." But the approach, which Heberlein likens to jumping off a cliff, "has really paid off," she says. In addition to the cAMP connection, Heberlein has evidence that lmo is also involved in cocaine sensitivity in mice.

Perhaps, given Heberlein's penchant for adventure, the cliff-diving method should come as no surprise. "Bold and daring things appeal to her," says Bargmann. Tijan agrees: "Ulrike ... takes risks. She goes for the throat. She tries things that other people haven't tried. She's a very strong person. You have to be to start something that other people think is bound to fail."

"I really admire where the work has gone," adds Bargmann. "It probably isn't even zany anymore. Which is too bad." ■

khopkin@the-scientist.com



This problem-based learning module includes the following:

- Description of the module
- Learning objectives
- Student Handout (the "problem" handed out to students)
- Content (contains the answers to the problem; handed out after students found their own answers)
- Glossary
- Resources

This module, along with 5 additional ones, can be found at:
www.thepepproject.net

Steroids and athletes: Genes work overtime

Description of the module

The use of steroids by athletes (and body builders) is common and it presents serious health risks. Despite the potential disqualification of athletes for using steroids before or during competition, athletes continue to use them. They must feel that the advantage of enhanced performance is worth the risk of being disqualified. In this module we explore the mechanism by which steroids promote muscle growth. They are notorious regulators of gene transcription, resulting in the synthesis of muscle proteins. Athletes who use steroids try to outwit the drug-testing "police," but, often, they fail the drug test. In this module, we highlight why steroids can persist in the body long after the person stops using the drug.

Learning objectives

1. Understand what is a steroid
2. Understand the definition of 'anabolic' and 'androgenic'
3. Understand the concept of a lipophilic molecule
4. Understand the difference between passive and facilitated diffusion
5. Understand the concept of hormone receptors and where in a cell they are located
6. Understand how proteins pass through a nuclear membrane
7. Be able to describe the structure of DNA
8. Understand the process of gene transcription, how it can be "turned on"
9. Understand the process of protein synthesis
10. Be able to identify parts of a muscle cell; understand how muscle contraction occurs
11. Understand the role of the liver and the kidney in eliminating compounds from the body

This module integrates information from the following areas:

cell biology, endocrinology, chemistry, physiology, sociology, sports

Student Handout

It's fairly common knowledge that exercising a muscle makes the muscle grow and become stronger. However, athletes in the US as well as in many other countries try to enhance their muscle performance even more by using steroids. Similarly, body builders use steroids to make their muscles grow beyond the size that would be produced naturally by lifting weights. How does this actually happen, and what are the consequences?

Let's explore the biology of steroids. Steroids are compounds that are synthesized in the body from the precursor, cholesterol. Some steroids are made in the adrenal glands (near the kidney) and some are made in the sex glands of both males and females.

1. Give an example of a steroid found in the adrenal glands and in the male and female sex glands.

2. Which of these steroids is used by athletes and by body builders?

3. What is an anabolic steroid?

Although anabolic steroids are used to increase muscle growth (and enhance performance), they do a lot of other things in the body as well. In fact, all anabolic steroids have androgenic properties, despite claims to the contrary. For this reason, they are termed anabolic-androgenic steroids or AAS, although most people just say "anabolic steroids" as a shortcut.

4. What is an androgen?

5. List 3 common androgenic effects of anabolic steroids in the body.

When athletes use anabolic steroids to enhance their performance, they don't just take a pill or an injection before the race or before the game. They must use the drug over a period of time in order to obtain the muscle growth. To understand why this is the case, we need to know how steroids actually make the muscles grow. First, after taking an anabolic steroid (by mouth or by injection), the steroid enters the bloodstream and travels to all tissues in the body (see Module 1). Most anabolic steroids are very lipophilic, and therefore, they can cross cell membranes easily to reach the inside of all cells.

6. Define "lipophilic." What characteristic of the steroid structure makes it lipophilic?

7. Why can a lipophilic steroid cross cell membranes so easily?

Once inside the cell, the steroid binds to a special protein called a steroid receptor. In this case we are referring to the "androgen receptor." The complex containing the anabolic-androgenic steroid and its receptor then travels through the cytoplasm and crosses the nuclear membrane to enter the nucleus.

8. How does a big, bulky molecule consisting of a steroid and a protein get across a nuclear membrane?

Once in the nucleus, the steroid receptor complex comes into contact with the DNA. The steroid receptor binds to a specific site on the DNA molecule, causing the DNA to start the process of gene transcription. This leads to the synthesis of certain proteins, depending on the cell-type and the part of the DNA to which the steroid receptor complex binds. All of these events take time, and a sustained use of the steroid is required to continually instruct the genes to synthesize more protein.

9. What kind of molecule is DNA? Describe its essential features.

10. What is gene transcription?

11. How is the protein synthesized?

The cell-type that contains the androgen receptor will define the kind of protein that is synthesized. For example, in the case of the muscle cell, the anabolic steroid will stimulate the synthesis of certain types of muscle fiber proteins.

12. In what type of cells would anabolic-androgenic steroids act to cause:
    - the growth of chest hair
    - acne
    - emotional disturbances such as aggression

As more muscle fiber proteins are produced, the muscle gets bigger and more powerful. But once the athlete or body builder stops taking the steroids, their muscles slowly revert to their normal size.

13. Draw a muscle cell. Label the essential structures in the muscle cell that help it to contract. Which muscle proteins constitute the muscle fibers?

The use of the steroids provides an unfair advantage over non-drug using athletes. So steroids have been banned from use in local, national and international competition. Despite this rule, many athletes continue to use steroids until shortly before a competition, when they hope that a drug test will not detect it. In many cases, this plan doesn't work. The drug is still present in their bodies long after the athlete stops taking it. This is due to the lipophilic character of the steroid.

Lipophilic compounds are difficult to eliminate from the body (the same is true for THC, found in marijuana). Normally, drugs are eliminated by the liver and the kidney. Enzymes in the liver convert (metabolize) drugs into a more water-soluble (polar) form. Once the drug is in a more water-soluble form, it travels through the bloodstream to the kidney, where it is collected in the urine and eliminated from the body. But in the case of highly lipophilic drugs such as anabolic steroids, they are metabolized very slowly so that only small amounts of drug are eliminated over time.

14. Why is it so difficult for an anabolic steroid to be metabolized by the liver enzymes?

15. Why is it so difficult for an anabolic steroid to be retained by the kidney where it would be eliminated in the urine?

If the lipophilic steroid can't be metabolized easily nor retained by the kidney, then it re-enters the bloodsteam to circulate throughout the body. However, it does like to "hide" in cells that contain a lot of lipids, such as fat cells. With continued use, the anabolic steroid starts to accumulate in the fat cells.

16. If the athlete stops using the steroid, the amount of steroid in the blood starts to decrease. What would account for the initial decrease of steroid in the blood?

17. Although the steroid decreases in the blood, it doesn't disappear right away. Instead, there is a steady low-level amount of steroid that is present in the blood over a long period of time (and thus the positive drug test). Where is the steroid coming from? What is the major force moving it into the bloodstream?

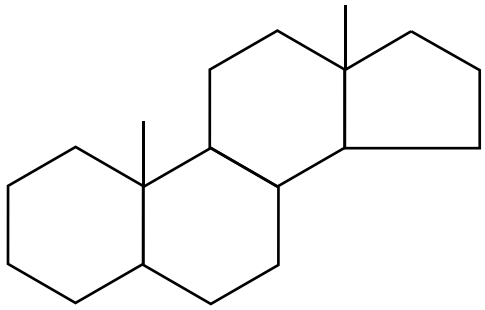
The ban on steroid use in sports is not based solely on the unfairness issue. There are serious health consequences that can occur from long-term steroid use.

18. List 3 additional health consequences from the repeated use of anabolic steroids.

The biochemistry of steroids

Steroids are a class of **hormones** that are synthesized by specific cells or tissues in the body and released into the bloodstream. Steroids are **non-polar** molecules produced from the precursor cholesterol. Four interconnected rings of carbon atoms form the skeleton of all steroids (**Figure 1**). The type of steroid formed is dependent upon the **polar** hydroxyl groups (OH) attached to the interconnected rings and the synthesizing tissue. Examples of synthesizing tissues, the corresponding steroids and some of their many functions are listed below.

Adrenal gland	glucocorticoids (cortisol)	maintain blood glucose during stress, anti-inflammatory
	mineralocorticoids (aldosterone)	regulate kidney function (water retention)
Ovaries	estrogen	promotes endometrial cell (uterine) proliferation
	progesterone	promotes endometrial cell differentiation
Testes	testosterone	stimulates sperm production promotes muscle growth



General steroid structure

Figure 1. The general structure of a steroid molecule is shown. Different steroids are defined by the location of polar hydroxyl groups (OH) attached to the C atoms within the rings.

Most steroids are used for medicinal purposes, especially the glucocorticoids, which are powerful anti-inflammatory agents. However, due to very serious side effects from long-term use (such as weight gain, bone density loss, increase in blood cholesterol levels, and liver disorders), they are only used as a last resort. Estrogen and progesterone are used in birth control pills and also in post-menopausal women to replace what is lost during aging (this is controversial). Testosterone (**Figure 2**) is an **anabolic steroid**, which promotes growth of muscle tissue. "Anabolic" literally means to build up tissue and it refers to the retention of nitrogen atoms in the body reflecting an increase in protein synthesis and/or a decrease in protein breakdown. While testosterone may be used in some clinical situations (e.g. testosterone-deficient men), it (or synthetic versions) is used mainly by body builders to increase muscle growth and by athletes to increase muscle growth and performance. Testosterone, like other steroids, has multiple effects in the body. It not only promotes muscle growth, it is also an **androgen.** It causes the development of male sexual characteristics such as growth of chest and facial hair, growth of the testes and deepening of the voice (Figure 2). Other effects of testosterone include acne, fluid retention, increased libido, aggression and other psychological disturbances.

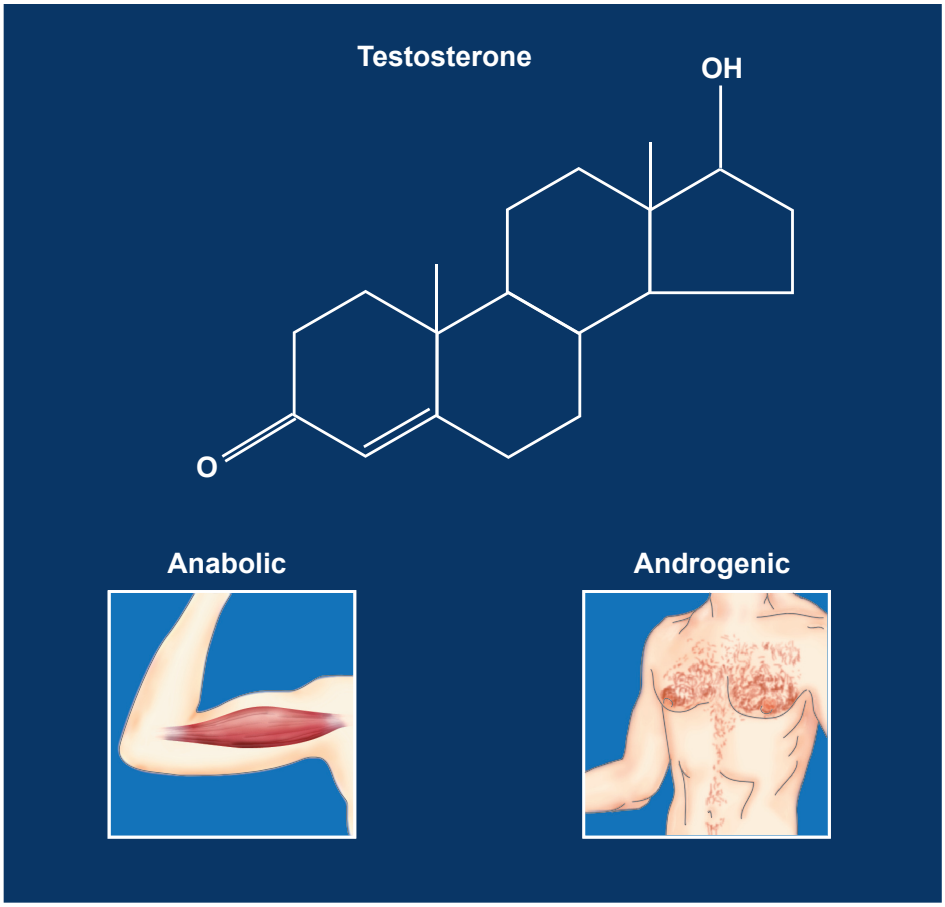


Figure 2. The structure of testosterone is shown. This steroid, synthesized in the testes, has both anabolic and androgenic properties.

Synthetic anabolic steroids

There are several problems with the use of testosterone. Since it has both androgenic effects (development of male sexual characteristics) and anabolic effects (promotion of muscle growth), both males and females may appear to be more "masculine." Athletes have tried to get around this issue by using synthetic forms of testosterone that have a chemical structure modified slightly from the original testosterone. These synthetic versions are called **anabolic steroids** and the manufacturers claim that they are more selective in their ability to produce anabolic effects compared to androgenic effects. However, despite these claims, anabolic steroids do have androgenic (masculinizing) effects, and thus a new terminology has emerged—anabolic-androgenic steroids, or AAS. The androgenic effects of anabolic steroids are a big problem for females who can develop facial hair, male pattern baldness and deepening of the voice (some of these effects are irreversible!). Another problem with taking the natural form of testosterone is that it is not very effective when given orally. After oral administration, testosterone is absorbed from the intestine into the bloodstream, which takes it to the liver (see Module 1), where it is immediately metabolized (inactivated). Thus, relatively little testosterone circulates throughout the bloodstream to reach its target. To address this problem, the chemists have chemically modified the testosterone structure to make it more difficult for the liver to metabolize it. The major chemical modification is the addition of C and H atoms (alkyl group) on the 5-membered ring at carbon #17 (**Figure 2**). Thus, this modification allows more testosterone to be available in the general circulation. However, there is a problem. The addition of an alkyl group at the 17th C atom not only enables the testosterone to be more slowly metabolized by the liver, but it also causes the liver to work harder to get rid of it, eventually resulting in liver damage or cancer.

How does an anabolic steroid reach its target?

Once in the bloodstream, the anabolic steroid travels to all tissues in the body, where it enters the cells to reach its target. In order to get into a muscle cell for example, the steroid must leave the capillary and then enter the muscle cell. This means that the steroid must cross two different types of membranes, the capillary membrane and the muscle cell membrane. To cross the capillary membrane, there are numerous pores or **fenestrae**, which allow small molecules to squeeze through (**Figure 3** and see Module 1). However the muscle cell membrane (like most cells in the body) does not have these small pores and therefore the steroid can only cross the membrane by diffusing across or by transport via a carrier protein. Steroids cross the muscle cell membrane by **passive diffusion**, which occurs in the direction of the concentration gradient— this does not require energy. Passive diffusion depends on the physiochemical characteristics of the membrane and the drug. The cell membrane, like all cell membranes in the body, is a lipid bilayer (**Figure 4**). It consists of lipids arranged with their polar head

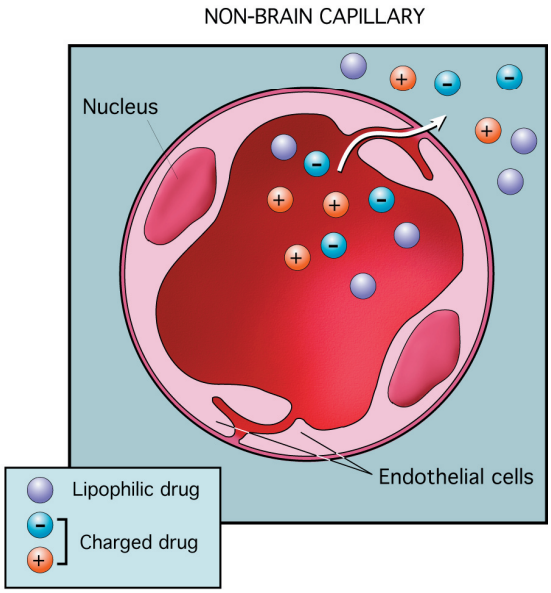


Figure 3. A capillary is composed of endothelial cells that connect together loosely. Small pores or *fenestrae* are also present, allowing solutes to move in and out of the capillaries.

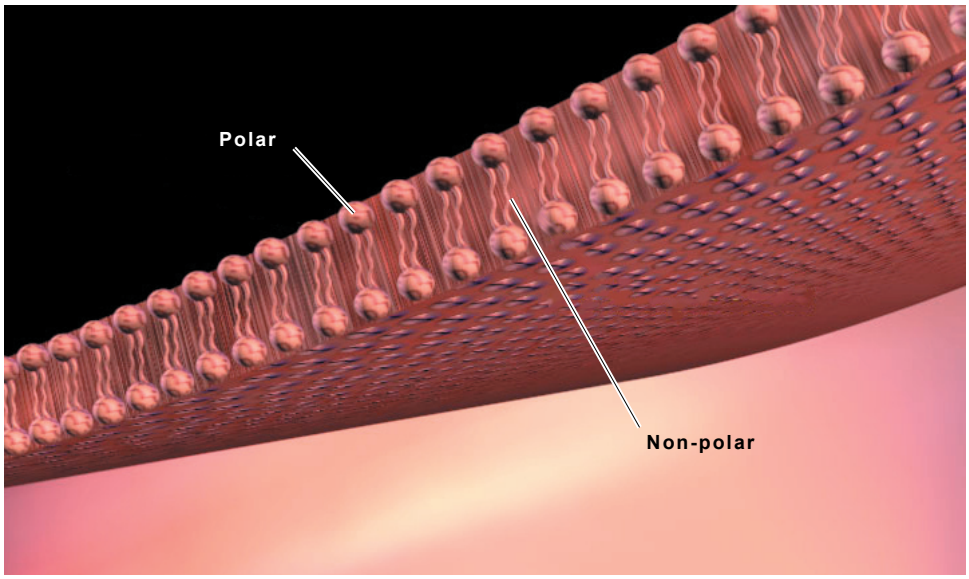


Figure 4. Schematic view of a cell membrane. Lipids are arranged with polar head-groups facing the outside and inside of the cell, while the fatty acid chains form the non-polar (hydrophobic) membrane interior.

groups facing the outside and inside of the cell. The chains of fatty acids face each other, forming the **hydrophobic** (water-fearing) or non-polar interior. Because anabolic steroids are very **lipophilic** (lipid-loving), they diffuse easily into the hydrophobic membrane interior. As they concentrate within the hydrophobic membrane interior, a new driving force is generated, pushing the steroid into the cytoplasmic side of the cell membrane. Once the anabolic steroid diffuses into the cytoplasm of the cell**,** it binds to the androgen receptor (**Figure 5**). [Receptors for other steroids are found in the nucleus instead of the cytoplasm.] This complex of steroid and protein then crosses the nuclear membrane to enter the nucleus of the cell, where it exerts its effects. In this case, passive diffusion can't occur because the protein is too large and not lipophilic. Instead, the steroid-receptor complex moves through small pores in the nuclear membrane to enter the nucleus. Although scientists are still elucidating exactly how this occurs, it is possible that the complex interacts with transport proteins that line the nuclear pores. This is an example of **facilitated diffusion,** which occurs in the direction of the concentration gradient. Therefore, no energy is required. This is unlike **active transport**, which occurs against the concentration gradient, and requires energy.

Steroids alter genetic function

Once inside the nucleus, the steroid-receptor complex binds to specific areas within the DNA (regulatory sites) to induce **gene transcription**, which directs the synthesis of specific proteins (**Figure 5**). A brief review of protein synthesis follows so we can understand how this happens.

DNA (deoxyribonucleic acid) is a large molecule containing the genes that code instructions for the synthesis of proteins. The code consists of a sequence of repeating subunits, or **nucleotides**. Each nucleotide has three parts: 1) a phosphate group (an acid), 2) a sugar (in the case of DNA, deoxyribose), and 3) a ring of carbon and nitrogen atoms (the nitrogen can form a bond with hydrogen so the nucleotide is basic) (**Figure 6**). A chain of nucleotides (**nucleic acids**) is formed by linking the phosphate group of one nucleotide to the sugar of an adjacent nucleotide. The bases stick out from the side of the phosphate-sugar backbone. The 3rd component described above, the base consisting of a ring of carbon and nitrogen atoms, occurs in 4 forms for DNA. These bases can be divided into two classes: the **purine bases** (adenine and guanine), which have double rings of nitrogen and carbon atoms, and the **pyrimidine bases** (cytosine and thymine), which have only a single ring. A molecule of DNA consists of two polynucleotide chains coiled around each other in the form of a double helix (**Figure 6**). The chains are held together by hydrogen bonds (see Module 5) between purine and pyrimidine bases – specifically, adenine is paired with thymine and guanine is paired with cytosine. Thus, one chain in the double helix is complementary to the other.

DNA is "read" by using three-base sequences to form "words" that direct the production of specific amino acids. These three-base sequences, known as triplets, are arranged in a linear sequence along the DNA. Each triplet codes for the synthesis of an amino acid and the specific chain of amino acids builds a specific protein. Most of the DNA is contained in the nucleus of the cell (a small amount is in the mitochondria), yet most protein synthesis occurs in the cytoplasm of the cell. Since DNA molecules are too large to pass through the nuclear membrane into the cytoplasm, a message must carry the genetic information from the nucleus into the cytoplasm. This message is carried by **messenger RNA** (mRNA; ribonucleic acid) molecules (Figure 5). The passage of information from DNA to mRNA in the nucleus is called **transcription** because the DNA sequence is actually transcribed into a corresponding RNA sequence. Once the mRNA passes through the nuclear membrane into the cytoplasm, it directs the assembly of a specific sequence of amino acids to form a protein – this process is **translation** (Figure 5). This occurs on ribosomes or in the rough endoplasmic reticulum (not shown in the figure). Thus, the synthesis of a protein is governed by the information in the DNA – mRNA simply serves as the messenger (and thus its name)! In the case of anabolic steroids, the steroid-receptor complex induces genes

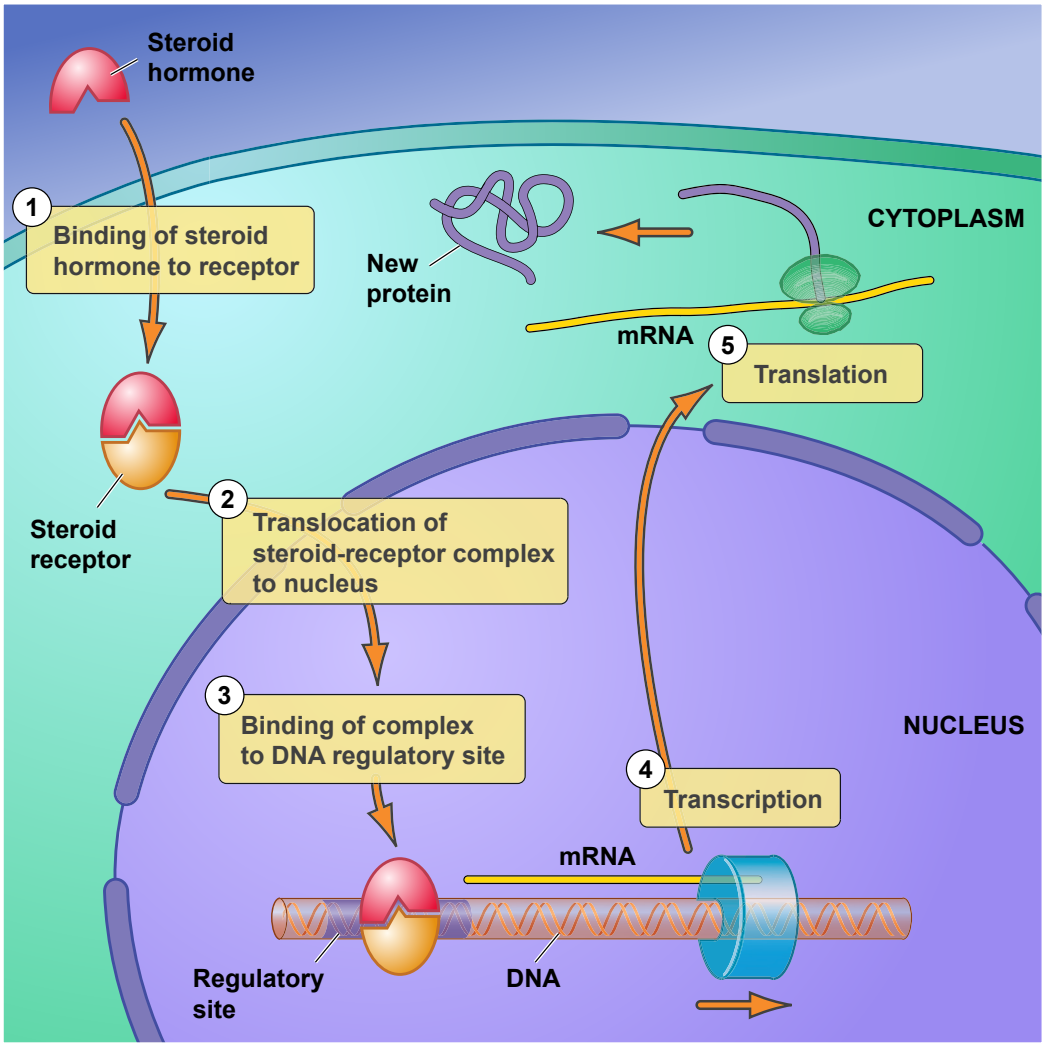


Figure 5. Testosterone (or anabolic-androgenic steroids) binds to the androgen receptor in the cytoplasm and the complex moves into the nucleus where it interacts with DNA to initiate protein synthesis.

to make specific proteins within muscle cells that help them to become larger and more powerful (discussed below). However, increased muscle growth is not the only action of anabolic steroids. Like testosterone, anabolic steroids can stimulate chest hair growth and cause acne and emotional problems (i.e., depression and hostility). The ability of anabolic steroids to produce these side effects is due to the cell type in which the steroid receptors are found and the specific DNA sequence that is transcribed. Thus, androgen receptors must be plentiful in cells of chest hair follicles (see Module 2), in secretory cells of sebaceous glands, and on neurons within the limbic system (important in mood) of the brain.

How does the alteration of genetic function by anabolic steroids increase muscle mass?

Consider the swimmer or weight-lifter who might use anabolic steroids (in fact both swimmers and weight-lifters in the 2000 Olympics were disqualified for steroid use). They have larger, more powerful arm muscles due to an increased production of specific proteins contained within skeletal muscle. A review of muscle structure will help us understand how this happens.

There are three main types of muscle in the body – skeletal, smooth, and cardiovascular. Steroids work predominantly on skeletal muscles, which account for approximately 40% of the 630 muscles in the human body!! Skeletal muscle cells contain a contractile mechanism that is activated by an electrical impulse generated when the neurotransmitter, acetylcholine, binds to acetylcholine receptors on the

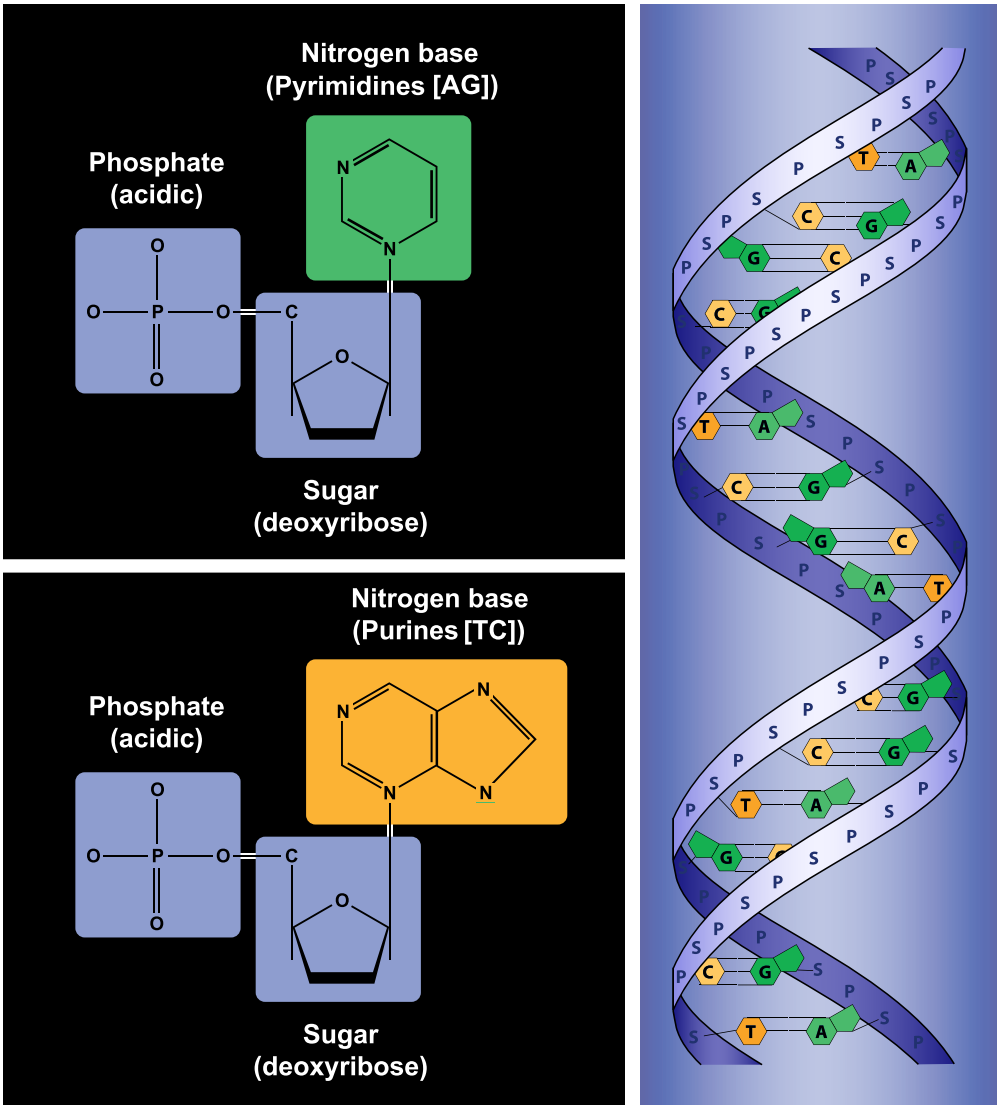


Figure 6. The generic structure of a nucleotide is shown. Nucleotides are joined together in a chain (phosphate groups of one nucleotide are linked with the sugar moiety of an adjacent nucleotide). The bases in one chain bind to complementary bases in another chain to form the double helical structure of DNA.

muscle (see Module 4). A single skeletal muscle cell is known as a muscle fiber (**Figure 7**). The term muscle refers to a number of muscle fibers bound together by connective tissue known as tendons, which are located at each end of the muscle. Skeletal muscle fibers (cells) appear striated because of an organized arrangement of thick and thin protein filaments (**myofibrils**) within cylindrical bundles in the cytoplasm--these myofibrils fill up most of the cytoplasm and extend from one end of a fiber to the other end. Each myofibril contains a repeating pattern of the thick and thin filaments surrounded by the sarcoplasmic reticulum and the sarcoplasm (cytoplasm). One unit of this repeating pattern is called a **sarcomere** (**Figure 8**). The thick filaments are composed of the contractile protein **myosin** and the thin filaments are composed of the contractile protein **actin**. Contraction occurs when the sarcomeres shorten by the action of the myosin filaments sliding over the actin filaments. The sliding of the myosin filaments is initiated when acetylcholine binds to its receptor in the muscle cell, generating an electrical signal to release calcium from the sarcoplasmic reticulum (where it is sequestered) into the sarcoplasm. The muscle relaxes when the calcium is removed from the sarcoplasm back into the sarcoplasmic reticulum by the enzyme calcium-ATPase.

For advanced students:

The cellular mechanism for muscle contraction
Actin contains active sites to which myosin binds during contraction. When the muscle is relaxed, the active sites are covered by another protein called tropomyosin, preventing any contraction. Troponin molecules are located along the actin-tropomyosin filaments and they help position the tropomyosin filaments over the active sites on the actin filaments. When calcium enters the sarcoplasm, troponin undergoes a conformational change that results in the movement of tropomyosin off the active sites, allowing myosin and actin to interact. The uncovering of the active sites allows myosin heads to bind to the actin active sites, initiating a movement of the myosin head toward the center of the sacromere. This pulls the actin along and shortens the sarcomere, thus causing the contraction. Each of the myosin heads operates independently of the others, each attaching and pulling in a continuous alternating ratchet cycle. This cycle stops when the calcium is removed from the sarcoplasm (as described above), causing troponin to change its conformation back to the resting state. The tropomyosin can then "cover up" (rebind to) the active sites causing the muscle to relax.

Anabolic steroids will induce the genetic machinery (as discussed above) in muscle cells to synthesize more muscle proteins. More contractile proteins make the muscle cell bigger, and therefore, the whole muscle gets bigger. Muscle growth is aided by another important action of anabolic steroids. Anabolic steroids can also bind to glucocorticoid receptors (there is some similarity in the structure of androgen and glucocorticoid receptors), preventing glucocorticoids from carrying out their normal **catabolic** or muscle-breakdown activity. Athletic performance improves as the muscles grow. The performance-enhancing effects of anabolic steroids do not occur in people who are not exercising unless large doses are used. Athletes tend to believe that the more steroids they take, the bigger their muscles will become. However, this doesn't happen. There are only a finite number of steroid receptors in the muscle cell. Thus, when all of the receptors are bound to the steroid (i.e., the receptors become saturated), any additional steroid molecules remain in the bloodstream, where they travel to the liver and kidneys. The high levels of steroids presented to the liver and kidneys can cause damage. High doses of anabolic steroids can have other adverse effects too. They can actually increase protein breakdown during the muscular stress that occurs with intense athletic training, increase fluid and electrolyte retention, or produce an increase in body weight.

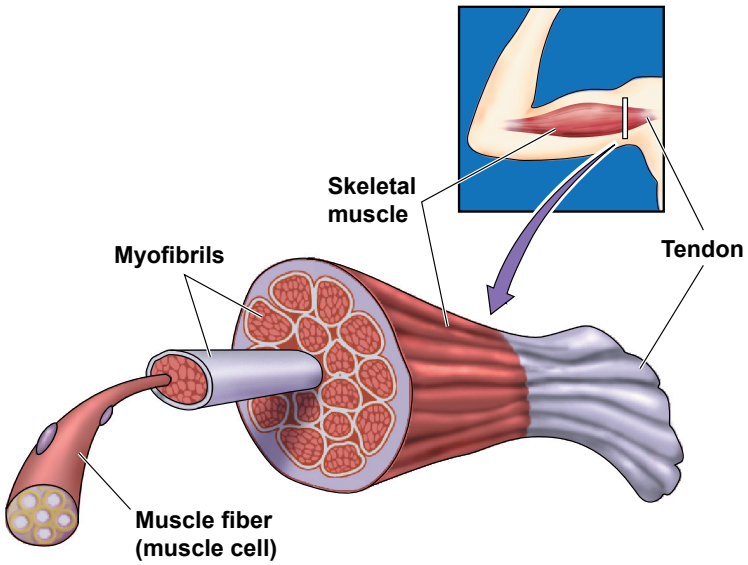


Figure 7. A skeletal muscle cell (also called a muscle fiber) is shown containing several myofibrils. These protein filaments are important in muscle contraction.

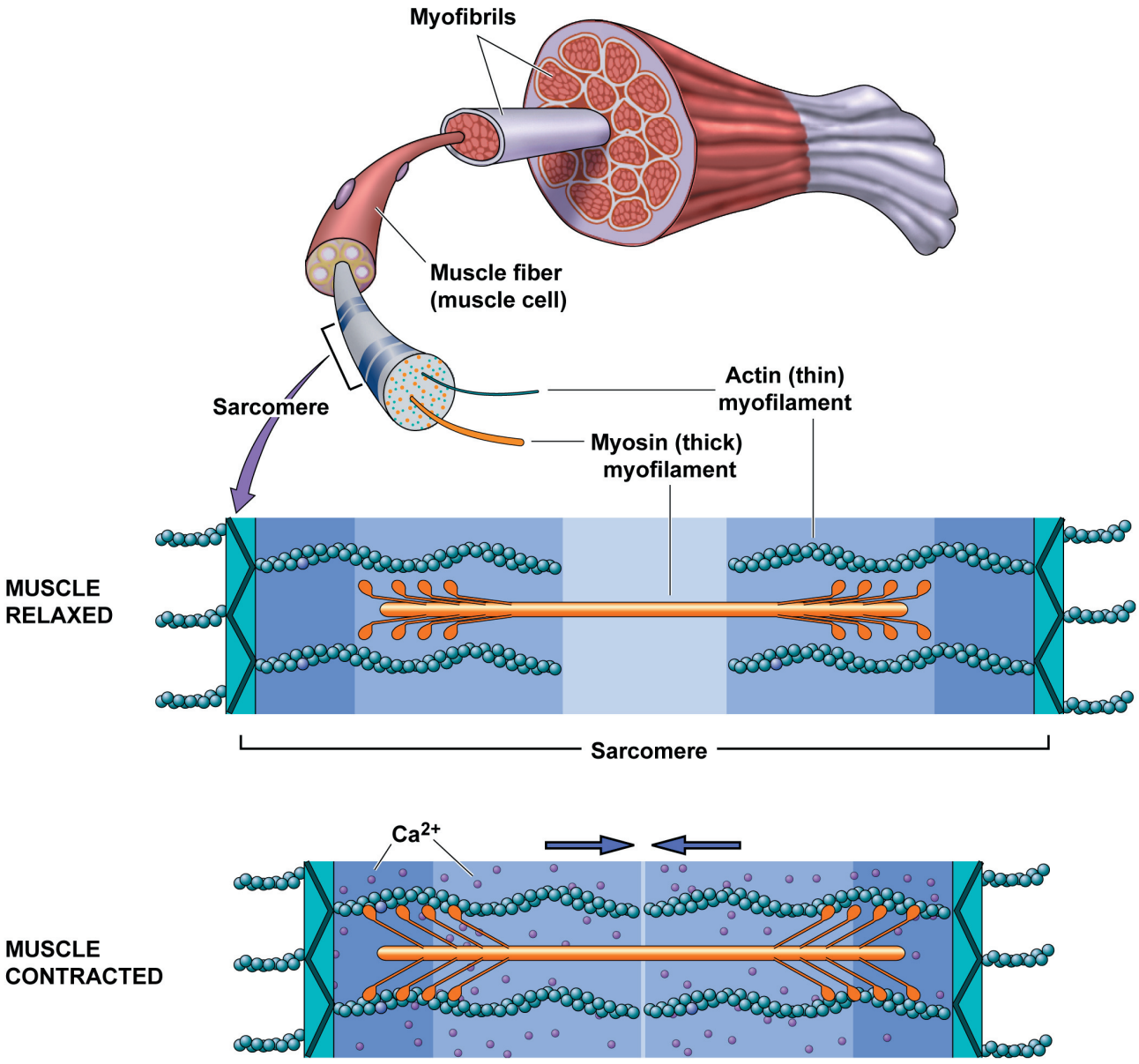


Figure 8. The repeating pattern of thick and thin filaments is a sarcomere. The presence of calcium causes sarcomeres to shorten when actin filaments slide over the myosin filaments. This produces muscle contraction. (After Seeley, et. al. Essentials of Anatomy and Physiology. Boston, MA: Mc-Graw-Hill, 1999.)

Why can anabolic steroids be detected in the body for long periods of time?

Every athlete knows that his/her urine will be tested for drug use when they enter an important competition. To avoid detection of steroids in their urine, athletes will stop using the drugs well before the competition. Yet, in many cases, steroids can still be detected long after the athlete stops using them (even weeks later!). The reason for this lies in the chemical structure of the anabolic steroid. As discussed above, anabolic steroids are very lipophilic molecules. This property makes it very difficult for the drug to remain long enough in the liver to be metabolized (inactivated) or in the kidney to be excreted in the urine (**Figure 9**). The lipophilic drug moves with its concentration gradient from the liver or the kidney cells right back into the bloodstream. Thus, it doesn't get eliminated very well. Instead,

it seeks out fat cells that exist in the athlete (these are much smaller than those found in the average couch potato!). The lipophilic steroid likes to enter fat cells, and with repeated use, the steroid accumulates there. When the user stops taking the steroid, the blood levels decline rather quickly in the absence of the drug. But now, the steroid concentration inside the fat cell becomes greater than that in the blood, so the concentration gradient reverses in the direction of fat cell-to-blood capillary. The fat cells are like a storage depot, releasing small amounts of steroid into the blood over time (via passive diffusion). Eventually the steroid gets metabolized and makes its way to the kidney to be excreted in the urine. This explains why it is possible to detect small amounts of the steroid in the urine at competition weeks after the athlete stops using it.

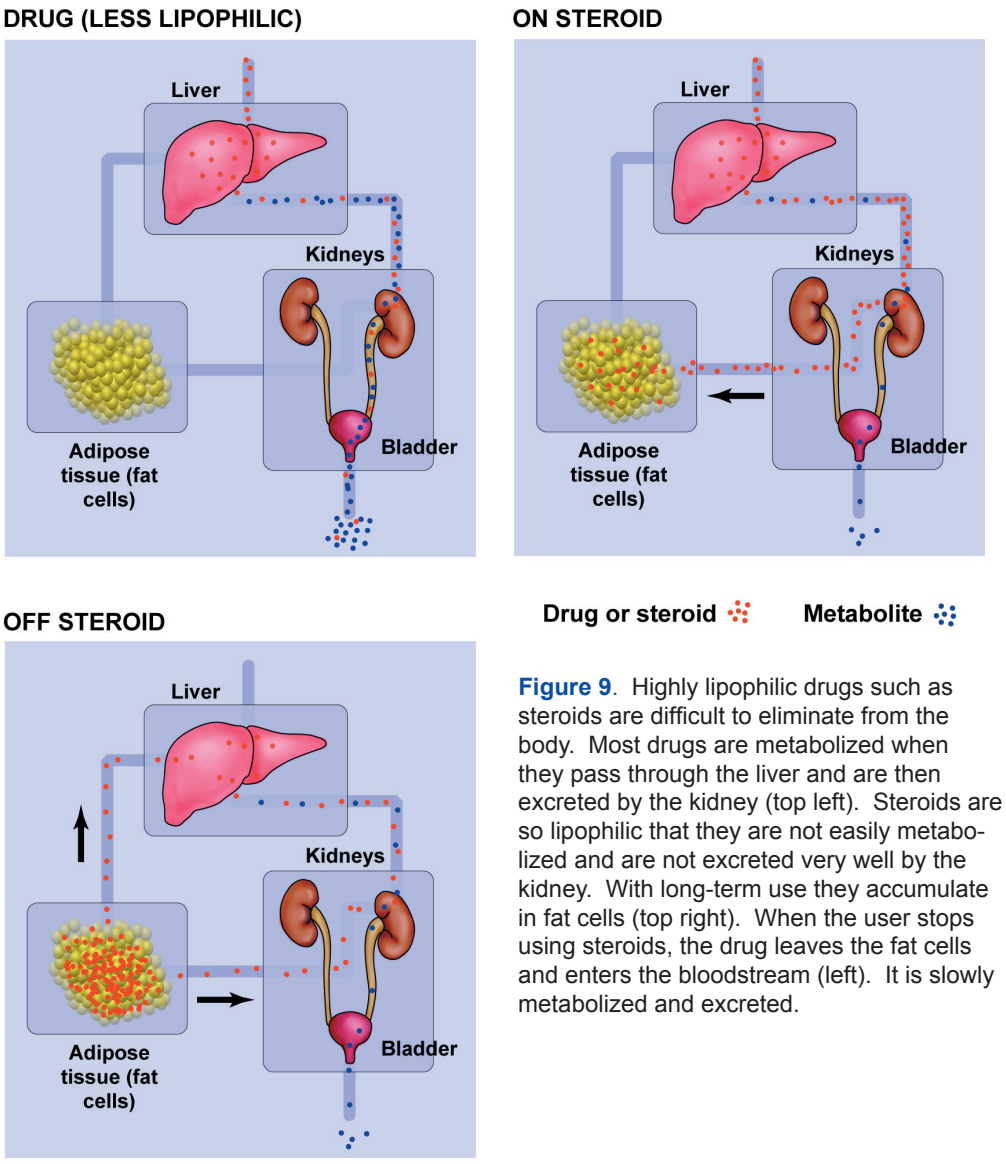


Figure 9. Highly lipophilic drugs such as steroids are difficult to eliminate from the body. Most drugs are metabolized when they pass through the liver and are then excreted by the kidney (top left). Steroids are so lipophilic that they are not easily metabolized and are not excreted very well by the kidney. With long-term use they accumulate in fat cells (top right). When the user stops using steroids, the drug leaves the fat cells and enters the bloodstream (left). It is slowly metabolized and excreted.

Why can't users stop taking steroids abruptly? Is it an addiction?

The answer is "not really." An **addiction** to a substance indicates that the person uses the drug compulsively, with a loss of control in their intake, despite negative consequences. Most athletes are not compulsive users of steroids (although there may be a few out there!)—if they were, they would not be able to stop taking the steroids prior to competition. However, chronic users can become dependent on steroids. A **dependence** means that the athlete's body adapts to the presence of the steroid, and if the steroid is withdrawn suddenly, physiologic symptoms emerge. Withdrawal symptoms include

nausea, headaches, dizziness, increased blood pressure, decreased libido (sex drive), depression and craving. The basis for this dependence involves the brain and the gonads. More specifically, the hypothalamus, found at the base of the brain, releases hormones that direct other tissues in the body to produce steroids (**Figure 10**). In the case of the sex steroids, the hypothalamus produces a hormone called "gonadotropin releasing factor" or GNRH. This hormone binds to GNRH receptors on the pituitary gland (located near the hypothalamus, but not actually part of the brain), where it activates the release of lutenizing hormone (LH) and follicular stimulating hormone (FSH). These pituitary hormones travel throughout the bloodstream and when they reach the gonads (i.e., testes and ovaries), they bind to LH and FSH receptors in gonadal cells to cause the release of testosterone and estrogen. The body attempts to keep the steroid levels in balance using "feedback regulation." When the sex steroid levels in the blood become elevated, the hypothalamus reduces its production of GNRH, the pituitary reduces production of LH and FSH, and the gonads reduce production of testosterone and estrogen. [In women taking birth control pills, this is the basis for the contraceptive activity—without enough LH and FSH, they can't ovulate.] So, when the athlete takes the steroids chronically, his/her hypothalamus stops producing GNRH and the gonadal tissues stop producing testosterone or estrogen due to this negative feedback. Now, if athletes stop taking the steroids abruptly, they won't have enough testosterone or estrogen. It takes some time (it can take 6 months!) for their hypothalamus, pituitary, and gonads to recover normal activity and start producing these hormones again. Therefore, all people who use steroids, even for therapeutic purposes, must taper off the drug slowly to give their hypothalamus, pituitary and gonads time to recover normal hormone production.

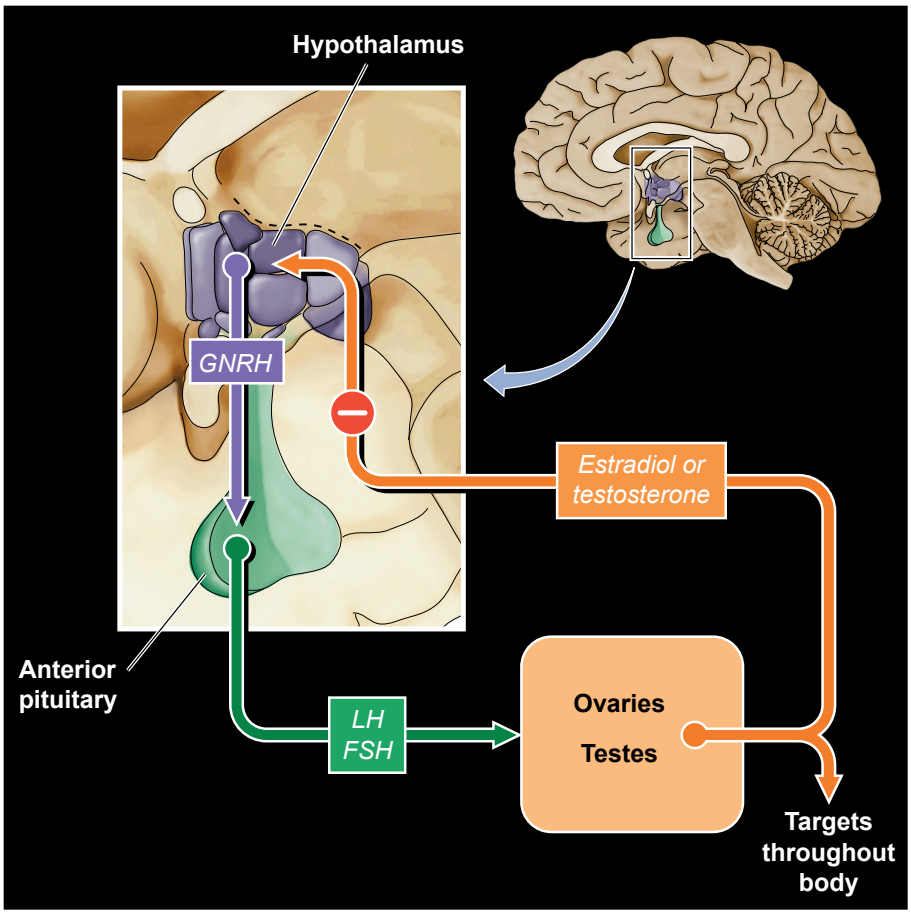


Figure 10. The hypothalamus-pituitary-gonadal "axis" is shown. GNRH is released by the hypothalamus, signaling the pituitary gland to release LH and FSH into the blood. In males, the testis synthesizes and releases testosterone in response to the LH and FSH. Circulating testosterone signals the hypothalamus to shut down GNRH release ("feedback inhibition").

Glossary

actin – contractile protein that is present in the thin filaments of the myofibrils.

active transport – the movement of molecules against the concentration gradient with the help of a transport protein. This transport requires energy in the form of ATP.

addiction – a behavior pattern that occurs when a person uses drugs compulsively, with a loss of control of their intake, despite negative consequences.

anabolic steroids – synthetic versions of testosterone designed to promote muscle growth without producing androgenic effects. The better term is anabolic-androgenic steroid.

androgen – a steroid hormone such as testosterone that is masculinizing (deepens voice, produces facial & chest hair, sperm production)

catabolic – a compound that causes the breakdown of muscle resulting in the net loss of nitrogen from the body. Glucocorticoids are catabolic in skeletal muscle.

dependence – the body functions normally in the presence of the drug. When the drug is present, the body has adapted physiologically to its presence. When the drug is removed, withdrawal symptoms are produced, usually in opposition to the effects produced by the drug's presence.

DNA (deoxyribonucleic acid) – a large molecule containing the genes that provide the instructional code for the synthesis of proteins. DNA consists of two complementary polynucleotide chains coiled around each other in the form of a double helix.

facilitated diffusion – the movement of molecules across a membrane with the concentration gradient. No energy is required, but transport proteins can become saturated, limiting the diffusion process.

fenestrae – small spaces or pores between endothelial cells that form the capillary membrane. These pores allow charged drugs or larger drugs to pass through the capillaries.

hormones – chemicals in the body that are synthesized in one tissue and secreted into the bloodstream for actions in tissues some distance away. They regulate many physiologic functions.

hydrophobic – "water-fearing"; a compound that is soluble in fat but not water. This is typical of compounds with chains of C atoms.

lipophilic – high lipid solubility. Lipophilic compounds dissolve readily in oil or organic solvent. They exist in an uncharged or non-polar form and cross biological membranes very easily.

messenger RNA – also known as mRNA or ribonucleic acid; it is transcribed from DNA and moves to the cytoplasm to direct protein synthesis.

myofibrils – a repeating pattern of thick (myosin) and thin (actin) protein filaments that are organized in cylindrical bundles within the sarcoplasm. The myofibrils extend from one end of a muscle fiber to the other end.

myosin – contractile protein that is present in the thick filaments of the myofibrils.

non-polar - a chemical property of a substance that indicates an even distribution of charge within the molecule. A non-polar or non-charged compound mixes well with organic solvents and lipids but not with water.

nucleic acid – a chain of repeating subunits of nucleotides.

nucleotides – the hydrolysis product of nucleic acids comprising 3 parts: 1) a phosphate group (an acid), 2) a sugar (deoxyribose for DNA and ribose for RNA), and 3) a ring of carbon and nitrogen atoms (nucleosides; purines and pyrimidines).

passive diffusion – the net movement of molecules from higher to lower concentrations. This form of diffusion does not require an energy source to occur.

polar compound – a chemical property of a substance that indicates an uneven distribution of charge within the molecule. A polar substance or drug mixes well with water but not with organic solvents and lipids. Polar or charged compounds do not cross cell membranes (lipid) very easily.

purine base – a type of nucleotide present in DNA that consists of double rings of carbon and nitrogen atoms. The two purine bases present in DNA are adenine and guanine.

pyrimidine base – a type of nucleotide present in DNA that consists of a single ring of carbon and nitrogen atoms. The two pyrimidine bases present in DNA are cytosine and thymine.

ribosomes – structures within the cytoplasm consisting of proteins and a different form of RNA (rRNA) that support the process of protein translation

sarcomere – one unit of a repeating pattern of actin and myosin present in a myofibril.

steroids – a class of hormones synthesized from cholesterol by specific cells in the body. They are powerful compounds that alter genetic function, causing numerous effects in the body.

transcription – the passage of information from DNA to mRNA in the nucleus; this is directed by several enzymes.

translation – the process of assembling a specific sequence of amino acids (based on the instructional code provided by mRNA) to form a protein. It occurs in the cytoplasm on ribosomes or in the rough endoplasmic reticulum.

Resources

The following resources provide supplemental information that pertains to the topic in this module.

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