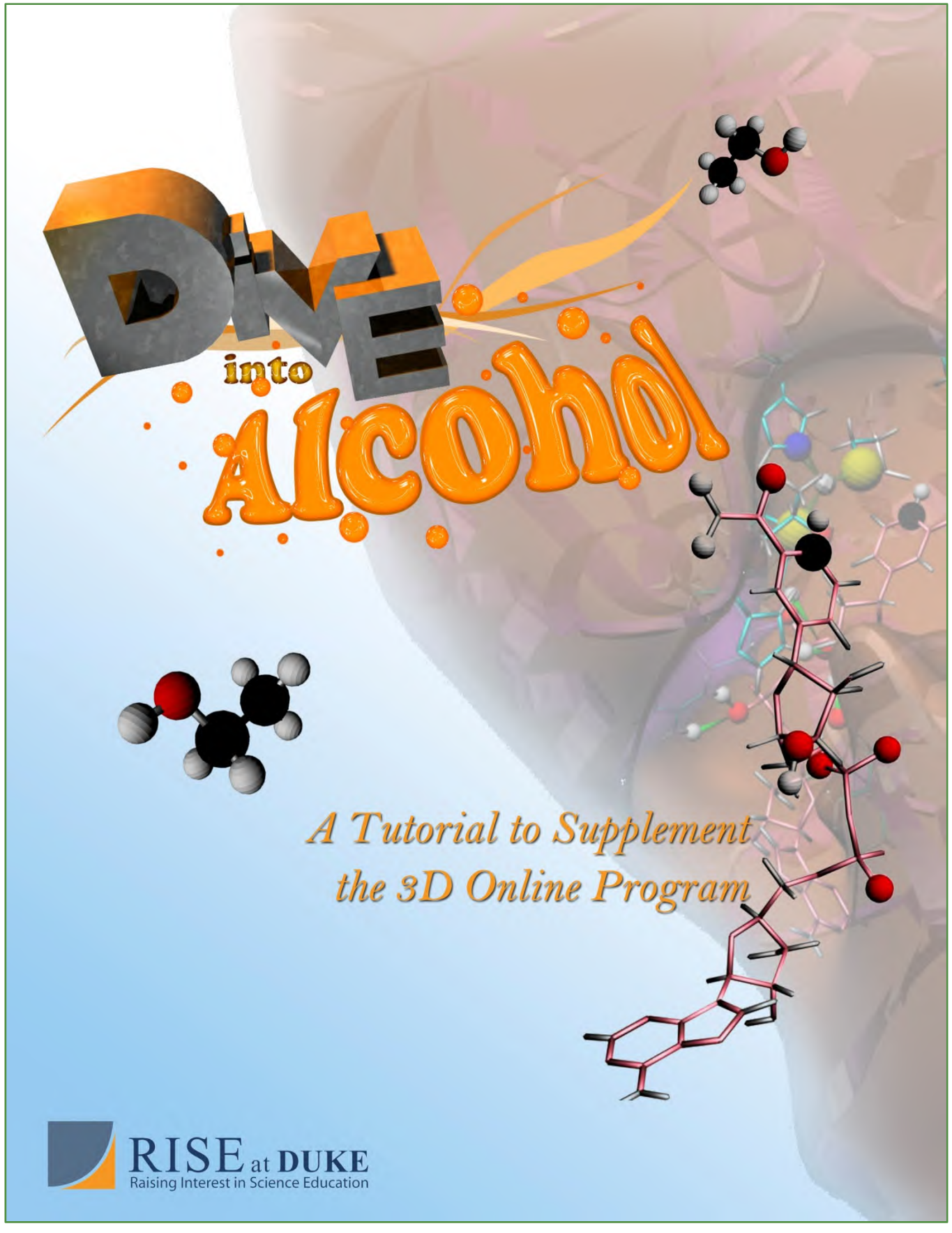
DIVE
into
Alcohol
A Tutorial to Supplement
the 3D Online Program
RISE at DUKE
Raising Interest in Science Education

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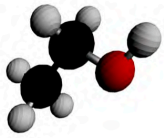

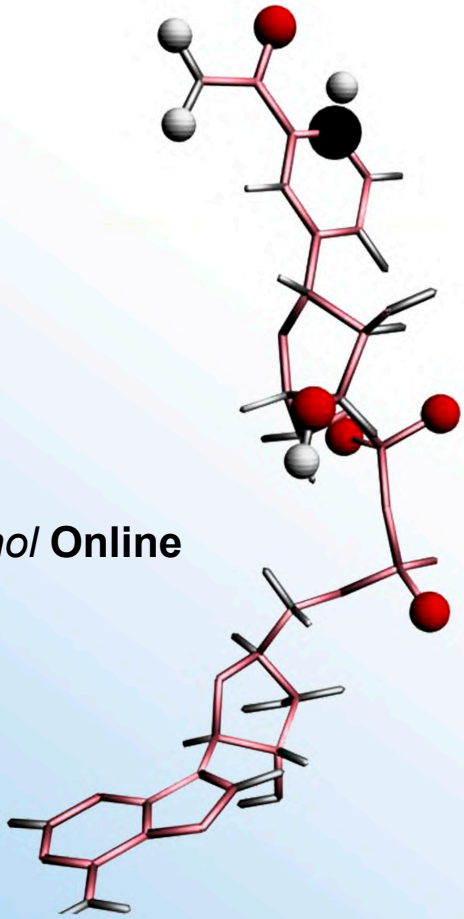

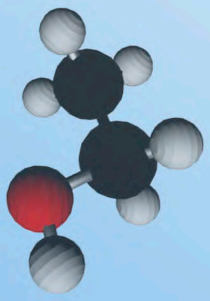

Introduction

What is *DiVE into Alcohol*?

DiVE into Alcohol is a 3D virtual reality experience to learn about the process of oxidation and the importance of gene polymorphisms on biochemical kinetics and alcoholism risk. The program was developed by a team of undergraduate students in an Independent Study in Science Education course at Duke, under the direction of Rochelle Schwartz-Bloom, Professor of Pharmacology, and Rachael Brady, Director of the Visualization Technology Group. Our goal was to develop the program as an online resource with the qualities of a video game and immersive feel. To build the program, we used virtual reality software that runs our Duke Immersive Virtual Environment (DiVE), a 6-sided virtual reality theatre.

DiVE into Alcohol can be can be accessed at www.rise.duke.edu/dive-alcohol. The program will run on any computer and browser after downloading the virtual reality plug-in software (free). All of the interactive features found in the actual DiVE are present in the online program using specific keyboard strokes. It's the next best thing to "being there".

This teaching tutorial complements the online version of *DiVE into Alcohol*. The tutorial provides a detailed discussion of specific chemistry and biology concepts that underlie the principles of alcohol absorption, distribution, and metabolism, including the influence of genetics on one's susceptibility to alcoholism. Bolded words are defined in the glossary at the end. The tutorial includes resources as well as a set of assessment questions to help the instructor assess students' understanding of the material. Answers are found in the Appendix. In addition, the Appendix contains the relevant National Science Education Standards that address the content contained in each chapter. The tutorial can be downloaded from our website at www.rise.duke.edu/dive-alcohol.

Using *DiVE into Alcohol* Online

To access the online virtual reality program, go to www.rise.duke.edu/dive-alcohol. Click on the first option of the 3 download links (DiVE into Alcohol Online). You will be prompted to read the instruction page (included below), print it out, and then press START. The first time the *DiVE into Alcohol* program is accessed, there will be an automatic download and installation of a virtual reality plug-in (free). AFTER a 1-2 minute wait the program will launch—please be patient! All successive launches will be instantaneous. Then the user MUST go through the 10 second tutorial to practice using the navigation keys on the keyboard (similar to navigation keys for a video game).

The introduction to absorption (first few minutes) does not have interactivity except for a segment that asks the user to 'walk around' the avatar. It is a good idea to practice moving around using the keyboard strokes during the first part of the program since the second part of the program involves several games requiring keyboard use.

The Duke Immersive Virtual Environment

The DiVE is a 3 meter x 3 meter room comprising walls, ceiling, and floor upon which computer graphics and animation are projected. An individual can walk into the theater and completely immerse him/herself in the 3D environment using "3D" glasses and a hand-held "wand" to interact with and navigate the virtual world (see photo below). For more information about the DiVE, please visit http://vis.duke.edu/dive.

To see a short movie of what it's like to be inside the DiVE and use the *DiVE into Alcohol* program, go to www.rise.duke/dive-alcohol and click on the 3rd option.

Students interact with alcohol molecules inside the DiVE.
Photo by Less Todd

Credits

Tutorial content developed by Megha Bisarya, Phil Tseng, David McMullen and Rochelle Schwartz-Bloom

DiVE into Alcohol cover design and logos created by Senmiao Zhan

Tutorial layout design by Dazhong Xuan

Tutorial header photos by Christine Adamczyk

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Using *DiVE into Alcohol* Online

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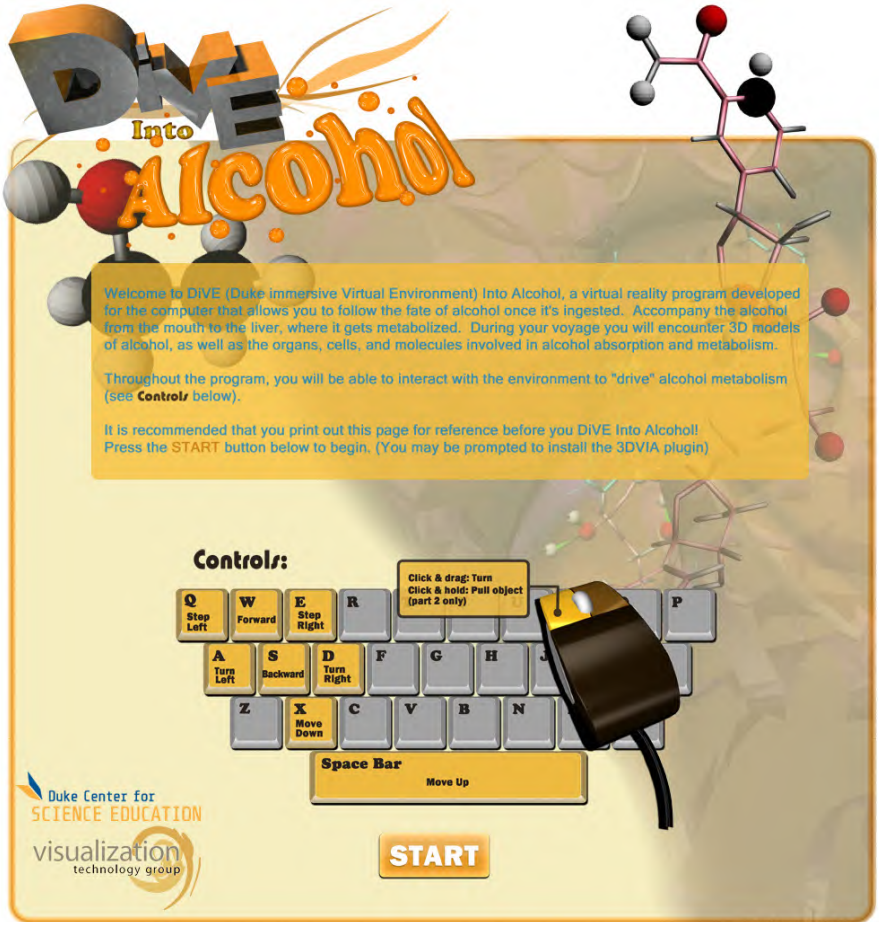
The Instruction Page



CHAPTER 1

Alcohol Absorption

LEARNING OBJECTIVES

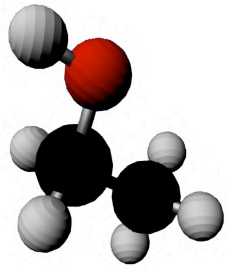
Students should be able to:

- *define compounds described as an alcohol*
- *describe the structure of ethanol and the basis for its polar character*
- *distinguish between filtration and passive diffusion*
- *explain why ethanol can move easily across biological membranes*
- *distinguish between epithelial and endothelial cells*
- *predict the effect of increasing expression of alcohol dehydrogenase (ADH) in the stomach on alcohol levels in the blood*

Alcohol is one of the oldest known drugs; however, scientists are still uncertain about its exact origin. Some historical evidence shows that it was most likely discovered when pre-agricultural humans accidentally drank liquids that had become contaminated with microbes. These microbes, such as yeast, undergo a metabolic process called **fermentation** when they live in **anaerobic** conditions. In the process of fermentation, sugars like glucose or fructose are **metabolized**, or broken down, into alcohol. Yeast ferment sugars until the alcohol concentration reaches about 15% by volume. Production of beverages with higher alcohol concentrations (*e.g.*, rum, vodka, whiskey) requires additional **distillation**, a physical process by which substances can be separated and purified.

Before learning how alcohol gets absorbed into the bloodstream, it is helpful to examine some of the physical and chemical characteristics of this interesting molecule. The structure of alcohol and its ability to dissolve in liquids and oils is what allows it to cross a biological membrane so easily. Let's review how this works.

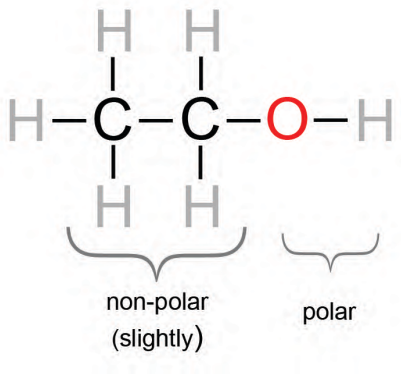
Alcohol structure



There are several different forms of alcohol, all of which share the common *hydroxyl group* (a bond between an oxygen and hydrogen shown as OH). The alcohol that is widely consumed as a beverage is known as **ethanol**, or ethyl alcohol, to scientists. Ethanol contains 2 carbon, 1 oxygen, and 6 hydrogen atoms. Other alcohols typically have more carbon atoms. The structural formula of ethanol, which shows how the atoms are arranged in the compound, is C2H5OH.

The “ball and stick” model of ethanol demonstrates the arrangement of 2 carbon (black), 1 oxygen (red), and 6 hydrogen (gray) atoms.

Alcohol solubility



The chemical structure of ethanol shows the non-polar hydrocarbon chain at one end and the polar hydroxyl group (O-H) at the other end.

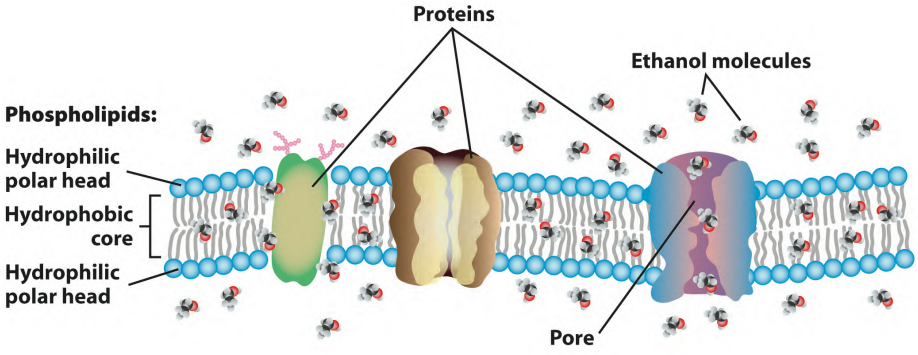
The presence of the hydroxyl group gives ethanol a polar character, which allows it to dissolve easily in water. It is polar because the oxygen atom is very electronegative. It “pulls” electrons from its neighbors, leaving them slightly positive. This creates a “pole” at that end of the ethanol molecule.

Another term used to describe a polar character is **hydrophilic**, or "water-loving." At the same time, the small carbon chain (2 carbons bound to 5 hydrogen atoms) imparts a slightly non-polar character to ethanol. This **hydrophobic** ("water-fearing") nature also allows ethanol to dissolve in non-polar organic solvents or lipids. Since the hydrocarbon chain is relatively short in ethanol, its hydrophobic character is eclipsed by the strong polarity and hydrophilicity provided by the hydroxyl group. However, as the length of the carbon chain increases, the alcohols become increasingly non-polar and progressively less soluble in water.

Crossing biological membranes

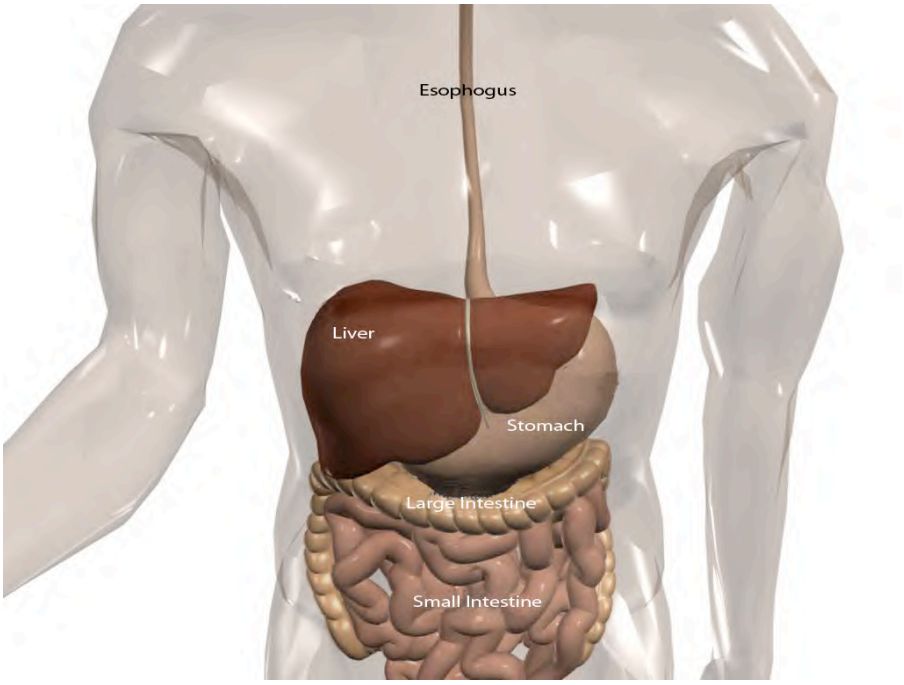
The physical and chemical properties of ethanol allow it to move effortlessly across biological membranes. Due to its small size and water-solubility, ethanol can move with water through tiny pores in the biological membrane; this movement, limited by size of the molecule, is called **filtration**. Filtration does not require any energy to move the ethanol molecules and it occurs in the direction of the concentration gradient. Additionally, because ethanol also has some non-polar character, it can

slip through the fatty or hydrophobic core of cell membranes. This movement occurs by **passive diffusion.** Like filtration, passive diffusion does not require any energy and it occurs in the direction of the concentration gradient. It is the balance of polar and non-polar characteristics that makes ethanol so unique in its absorption and distribution throughout our bodies.



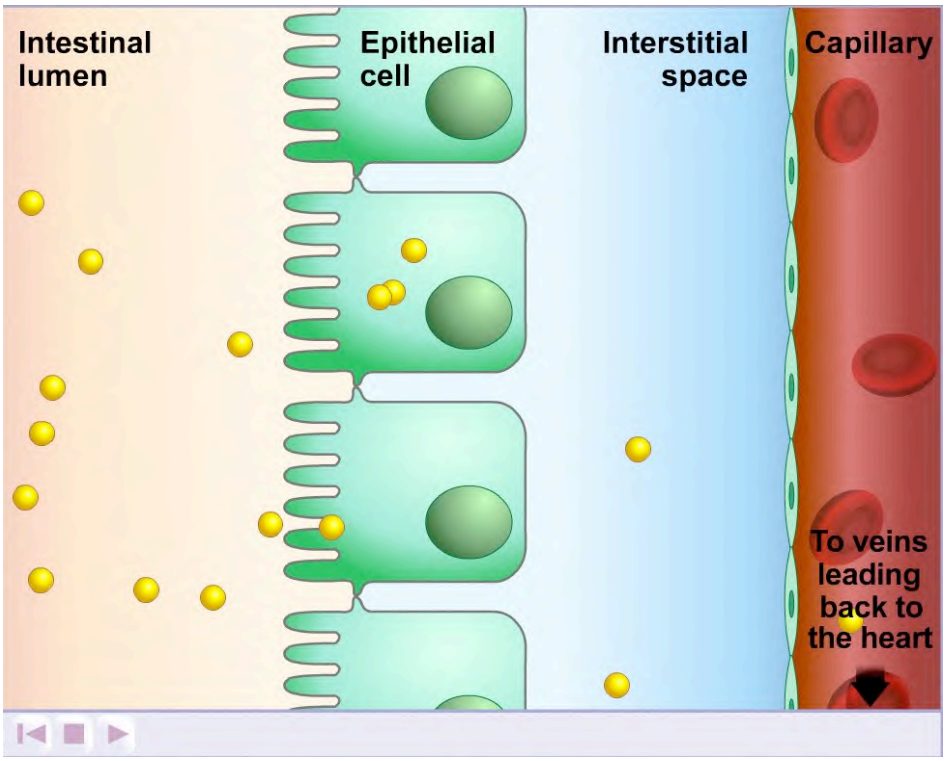
Ethanol molecules move through biological membranes by filtration (through pores and water spaces) and by passive diffusion through the membrane's lipid core.

Alcohol must cross 2 biological membranes to be absorbed into the bloodstream



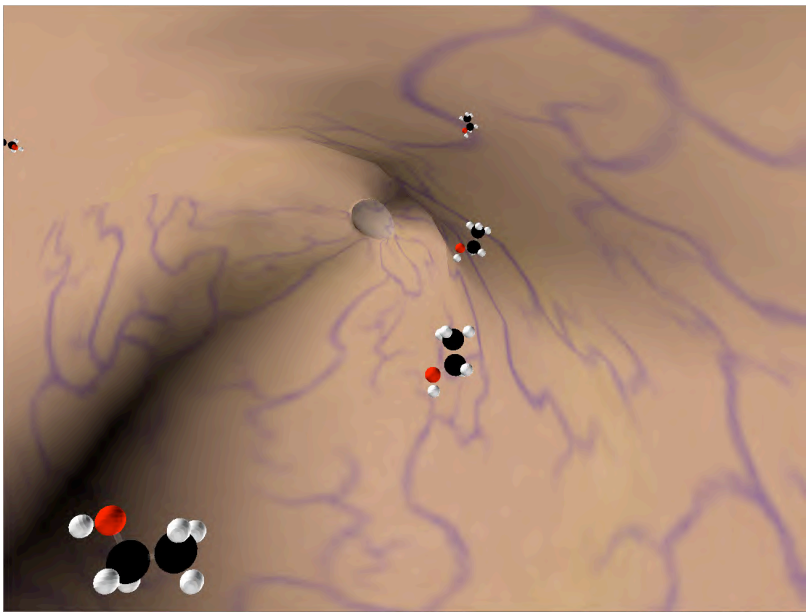
The GI tract is shown with the esophagus, stomach, small and large intestines.

To get into the bloodstream, the alcohol must cross two types of cell membranes. The first is the **epithelial cell** membrane, which lines the GI tract. On the other side of the epithelial cells lies the interstitial space. From there, the ethanol moves into the **endothelial cells** of the capillaries, the smallest vessels within the bloodstream. Each step in the absorption of ethanol occurs with the concentration gradient. While ethanol moves by passive diffusion and filtration through the epithelial cell membranes, most of the movement through the endothelial cell membranes of the capillaries is by filtration. The endothelial cells have a lot of water spaces between them and many "holes" to allow large nutrients into the capillary. So ethanol just filters easily through all of these spaces.



Alcohol molecules diffuse passively across epithelial cells of the small intestine and then move by filtration through endothelial cells into the capillaries. The movement occurs in the direction of the concentration gradient. To see an animation go to www.rise.duke.edu/apep

Food in the stomach slows alcohol absorption



Ethanol molecules in the stomach approach the opening created by the **pyloric sphincter** into the small intestine.

Food in the stomach delays gastric emptying--the process by which contents in the stomach are emptied into the small intestine. When food is presented to the stomach, a muscle at the bottom (the **pyloric sphincter)** closes off the exit of the food into the small intestine. Therefore, the food in the stomach can be digested by stomach acids before release into the small intestine. Since most ethanol absorption occurs in the small intestine, the presence of food in the stomach delays ethanol absorption into the bloodstream.

The type of food can delay ethanol absorption at different rates. For example, fat, which is high in french fries or peanuts, causes alcohol to remain in

the stomach longer. In the presence of fat, the hormone cholecystokinin is released from the stomach wall to delay gastric emptying. The extra time is needed to digest the fats inside the stomach.

On the other hand, it is widely believed that carbonated beverages can quicken alcohol absorption. Some have proposed that the presence of carbon dioxide (CO2) could physically expand the stomach and induce the pyloric sphincter to open the passageway into the small intestine. The belief is that the opening by the sphincter would relieve stomach pressure and allow the alcohol to enter the small intestine where it is rapidly absorbed. However, there is little scientific evidence to support the idea that CO_2 can cause the sphincter to open the hole.

You can travel with some ethanol molecules through the GI tract by viewing the online interactive virtual reality game that accompanies this tutorial. Go to www.rise.duke.edu/dive-alcohol.

Ethanol absorption differs in males vs females

The rate and extent of ethanol absorption from the stomach can differ depending on whether a person is male or female. There is an enzyme (a protein) called **alcohol dehydrogenase (ADH)** that metabolizes, or breaks down alcohol; it exists in the epithelial cells that line the stomach of males but not females. [Note: however, the gene for *ADH* is present in the stomach of both genders…see Chapter 4 about the *ADH* gene.] Therefore, in males, some of the ethanol (up to 30%!) is metabolized in the stomach before it ever gets into the bloodstream. So, for a given drink of alcohol, more ethanol is absorbed into the bloodstream of females compared to males; this explains, in part, the greater effects of ethanol in women compared to men. Interestingly, this sexual dimorphism is not detectable beyond age 50.

More information on ethanol metabolism can be found in Chapter 3.

CHAPTER 2

Alcohol Distribution

LEARNING OBJECTIVES

Students should be able to:

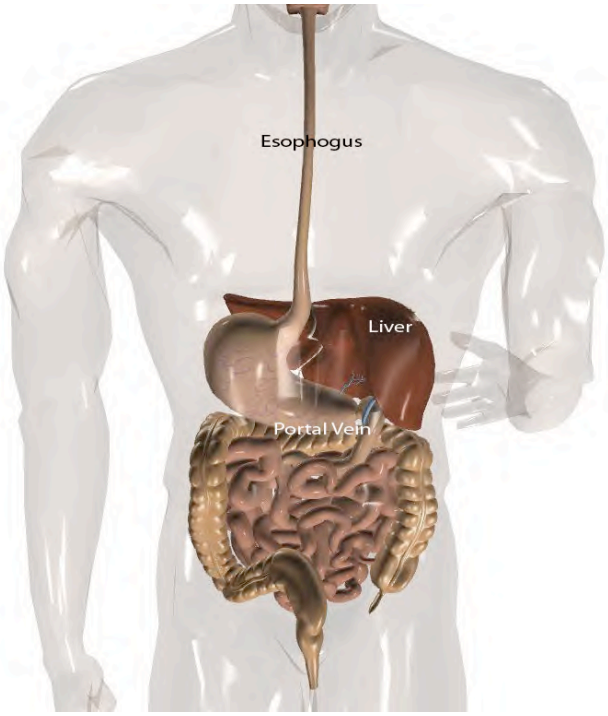
- *trace the path of a molecule from the stomach to the venous system*
- *trace the path of a molecule from the liver to the brain*
- *explain how ethanol reaches an equilibrium between the blood and tissues*
- *describe the relationship between volume and concentration*
- *explain why males and females of similar size will exhibit differences in the blood alcohol concentration (BAC) after drinking the same amount of alcohol*

Recall from the previous chapter that once ethanol reaches the small intestine, it moves through the epithelial cells that line the intestine, into the interstitial space, and then eventually moves into the capillaries, gaining access to the bloodstream.

Ethanol distributes throughout the body

Ethanol enters the bloodstream through capillaries, tiny blood vessels in the tissues of the body that carry blood from the arteries to the veins. The capillaries lining the entire GI tract converge into the **portal vein**, which carries deoxygenated blood to the liver. Once it reaches the liver, the portal vein then branches out into another capillary system. This system brings all the ethanol absorbed through the GI tract to the **hepatocytes**, the major cells of the liver. In these cells, ethanol is metabolized by enzymes into other molecules. Ethanol is primarily responsible for intoxication, while its metabolites have other effects (see Chapter 3).

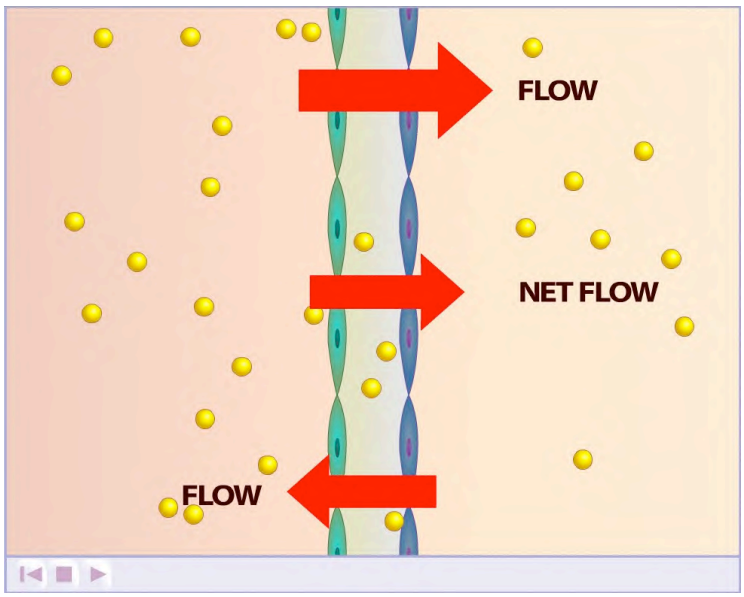
However, not all ethanol that enters the liver can be metabolized in the hepatocytes because there are only a finite number of enzyme molecules available to do the job. The ethanol that doesn't get



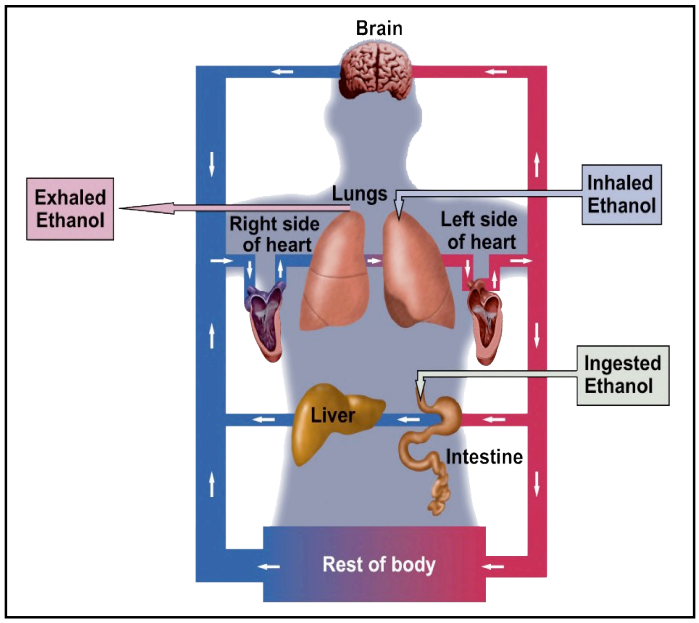
It's easier to see the portal vein from the back of the body. The portal vein carries blood (and the alcohol within it) from the capillaries in the GI tract directly to the liver.

metabolized while in the liver travels from the capillaries back into the venous system, which carries all deoxygenated blood into the heart (the right side). The heart then directs the blood into the lungs, where tiny amounts of ethanol are eliminated during exhalation. (This tiny amount is enough to estimate the **blood alcohol concentration** (BAC) by using a Breathalyzer™ test.) In the lungs, the blood is oxygenated, and still carrying the ethanol, returns it to the heart (the left side). Now the ethanol is distributed throughout the entire body via the arteries. Much of this blood, as well as the ethanol it carries, goes to the brain, where ethanol interferes with neuronal function and causes people to become intoxicated, or "drunk." For more information on how alcohol works in the brain go to www.rise.duke.edu/apep

The arteries bring ethanol to all tissues of the body where it diffuses down the concentration gradient from the bloodstream into the cells. When the concentration of the ethanol in the bloodstream relative t that in the tissues becomes constant, the system has reached a state of equilibrium. During equilibrium, ethanol molecules may continue to move back and forth between the bloodstream and the tissues; at this point, however, the rate of movement into the bloodstream equals that into the tissues, so there is no *net* movement in one direction or the other. Tissues with high blood flow, such as the brain, kidney, and lungs, will reach equilibrium faster than those that have low blood flow, such as skeletal muscle and tissue.



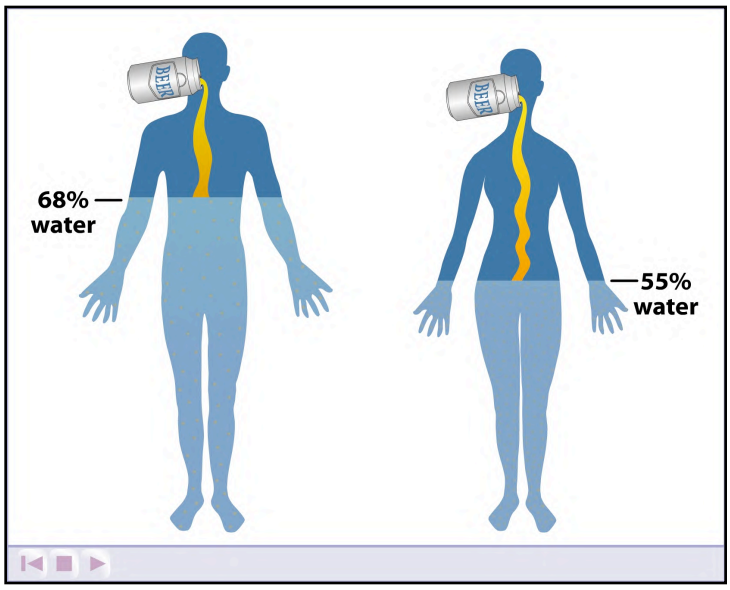
Eventually, ethanol reaches equilibrium between the blood and the organs to which it flows; when at equilibrium, there is no **net** movement of ethanol across the biological membranes separating the capillaries from the tissue cells. To see an animation go to www.rise.duke.edu/apep



Although ethanol enters the kidneys, less than 5% of it is excreted in the urine. Instead, the ethanol moves with its new concentration gradient (higher in the kidney than the blood) back into the capillaries. It then reaches the bloodstream and returns to the heart, to start the process of distribution over again. Each time it passes through the liver, some ethanol is eliminated by metabolism.

When ethanol is ingested, it travels from the GI tract to the liver. Some gets metabolized and the rest enters the major veins to go to the heart, where it is then pumped out to the brain and the rest of the body.

Food in the stomach slows alcohol absorption



Ethanol distributes into a smaller water space (i.e., blood) in females, causing a higher blood (and brain) alcohol level compared to males. To see an animation go to www.rise.duke.edu/apep

While ethanol distributes nearly everywhere throughout the body, almost none of it goes into **adipose tissue**, or fat cells. The majority of the alcohol molecules remain predominantly in water spaces such as the bloodstream, extracellular water, and intracellular water. Ethanol has a fairly polar nature due to its hydroxyl group (O-H) and its short hydrocarbon chain, so it favors polar environments over the fatty, non-polar environment of adipose tissue. This fits the “rule of thumb”: *like dissolves like*.

The total volume of water found in the body is called **total body water**. Males have a higher percentage of their body mass as water, while females have a higher percentage of their body mass as fat. Thus, if two individuals of opposite gender have equal body size and consume equivalent amounts of alcohol, the female will have a higher concentration of ethanol in her blood, since she has less water space into which the ethanol can distribute.

CHAPTER 3

Alcohol Metabolism

LEARNING OBJECTIVES

Students should be able to:

- *define oxidation*
- *describe what enzymes are and how they function*
- *name the two enzymes involved in the metabolism of ethanol*
- *name the crucial coenzyme required for most biological oxidation reactions*
- *name the two most important kinetic properties of enzymes*
- *describe the significance and meaning of these two properties*

Our bodies have a natural “protective” mechanism to eliminate drugs that are consumed; the elimination helps ensure that the duration of the drug’s effect is limited. Problems arise when the rate of elimination is slow, especially when one continues to take the drug. In the case of alcohol, one could remain intoxicated for hours after drinking several drinks. While this can be dangerous from a behavioral standpoint, the high levels of alcohol over time can also result in damage to several tissues in the body, most notably, the liver, heart, and brain.

Because ethanol is relatively polar, it can be eliminated from the body in any water environment, such as the breath, sweat, and urine. However, these routes of elimination are rather limited for ethanol, barely accounting for 5% of its total elimination from the body. Instead, we eliminate ethanol through a biochemical process called **metabolism**. Ethanol is converted to other, non-intoxicating, compounds primarily in the liver (but also in the stomach in males). This reaction involves **oxidation**, which is aided by specific proteins called **enzymes** that **catalyze**, or speed up the reaction. Before describing the metabolism (oxidation) of alcohol in more detail, it may be helpful to review the process of oxidation in general.

Defining oxidation and reduction (redox) reactions

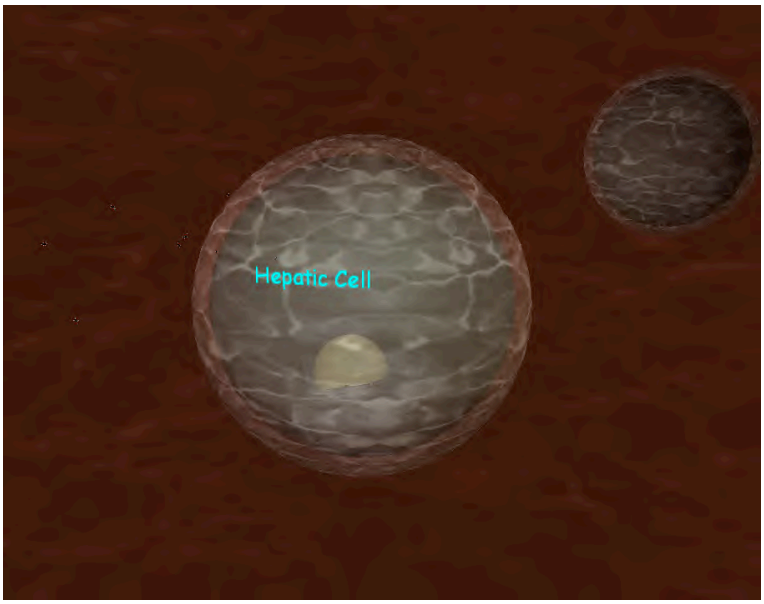
Oxidation is a process by which an atom, molecule, or compound loses one or more electrons. The loss of an electron can occur by losing a hydrogen atom, or gaining an oxygen atom (when oxygen binds to an element, the element loses the electron to the oxygen). The most common form of oxidation involves oxygen, as it can interact with a number of different substances, from metals to molecules in our cells. Oxidation leads to an increase in oxidation state. The **oxidation state** is the theoretical charge a molecule would have if all the bonds in the molecule were 100% ionic. Oxidation states are represented in numerical form and can be positive, negative or zero.

Reduction is the opposite of oxidation and leads to the decrease of oxidation state. It is a chemical reaction that occurs when electrons are gained by the addition of a hydrogen atom or loss of an oxygen atom (in this case, oxygen leaves the molecule without one or more of its electrons). Oxidation and reduction reactions always occur together and are referred to as **redox reactions**.

For more on redox reactions, see the Appendix.

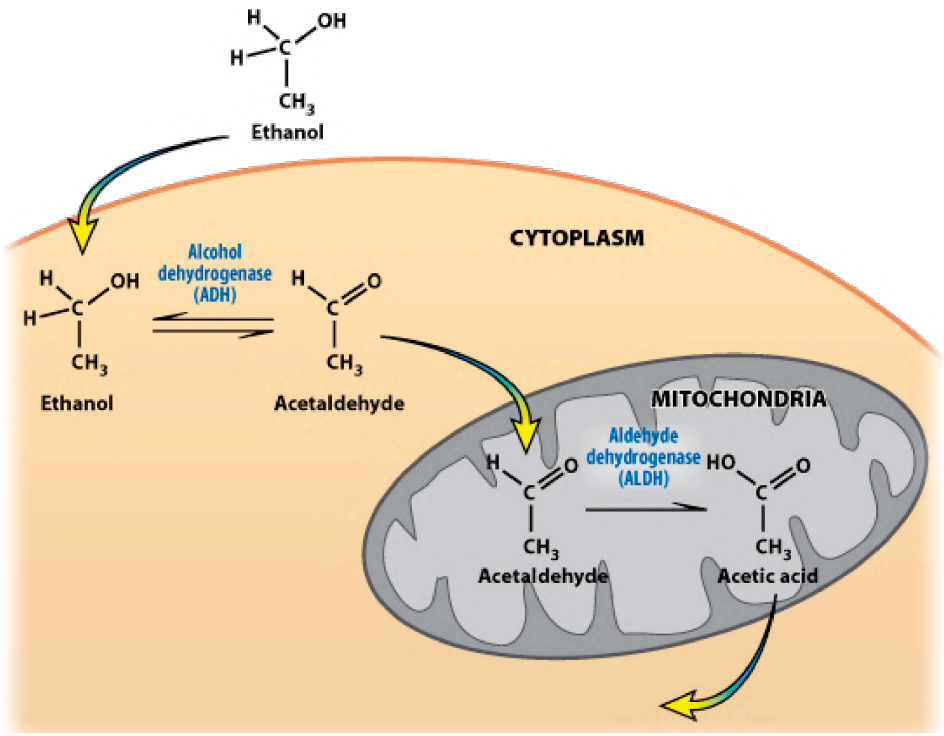
Ethanol is metabolized by oxidation in the liver

Metabolism of ethanol happens everywhere in body, but most of it happens in the liver, our metabolic powerhouse. Ethanol, like all other nutrients absorbed into the bloodstream from the intestines, travels to the liver before it goes to the heart and the rest of the body. Because ethanol is small and polar, it easily crosses the endothelial cells that make up the capillary membrane and then the membrane of the major cells in the liver called hepatocytes (or hepatic cells).



The major cells in the liver are hepatic cells; they contain thousands of specific enzyme molecules to metabolize drugs, hormones, and nutrients.

The hepatocytes are packed with two types of enzymes that are important for the metabolism of alcohols: **alcohol dehydrogenase (ADH)** and **aldehyde dehydrogenase (ALDH)**. First, with the help of ADH, ethanol is oxidized to **acetaldehyde**, a toxic compound, which can also cause cancer. [It is the acetaldehyde that causes the tissue damage mentioned above with long-term use of alcohol.] This reaction takes place in the cytosol of the hepatocyte because that is where the ADH is located. Then, to get rid of this toxic compound, ALDH helps to oxidize acetaldehyde to **acetic acid** (vinegar), a metabolite that is converted eventually to water and carbon dioxide (CO_2). The second reaction occurs mainly in the **mitochondria**, cellular organelles where most of the ALDH is found. The steps in metabolism are shown in the figure below.



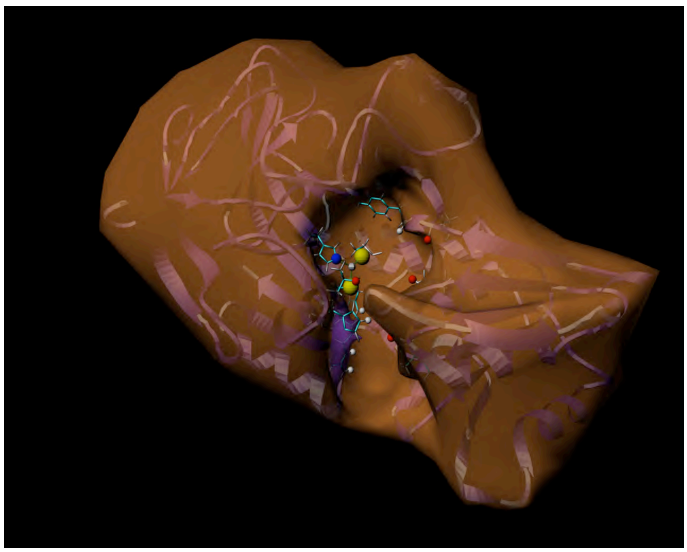
The cartoon shows that ethanol enters the hepatic cell cytoplasm and becomes oxidized with the help of ADH. The product acetaldehyde moves into the mitochondria where it gets oxidized with the help of ALDH.

Let's take a closer look at how the enzymes work.

Enzymes are specialized proteins that catalyze biochemical reactions. They participate in the reaction, but they are not consumed by it. In other words, enzymes are present in the same form before and after the reaction. The essential function of an enzyme is to increase the rate of a given reaction that would otherwise occur extremely slowly. In the present example, ADH increases the oxidation rate of ethanol.

Enzymes work in a "lock-and-key" fashion with their **substrates**, which are molecules on which the enzymes exert their biochemical function. In our example, one of the substrates is ethanol (the other is NAD^+, described below). Each enzyme has a characteristic 3-dimensional shape, usually with a cavity or pocket called the **active site** (the "lock"), into which the substrate (the "key") can fit. Molecules with shapes that are not compatible with the active site of the enzyme cannot be substrates. They can't bind to the enzyme—an analogy is the attempted insertion of an incompatible key into a lock.

When the substrate binds to the enzyme, it is called the "enzyme-substrate complex".



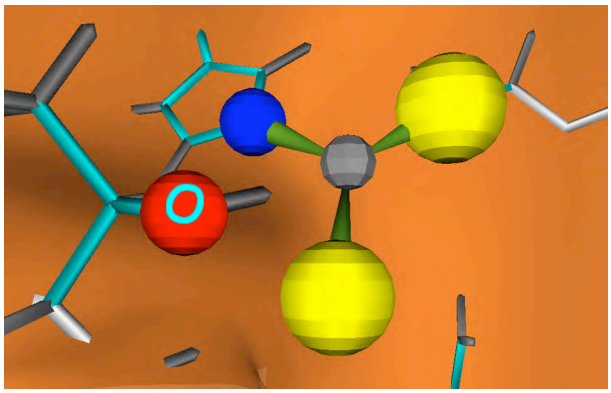
The chain of amino acids in the ADH protein twists and turns to form the 3-dimensional structure ("ribbon diagram"). If we place a "skin" over the ribbon, it is easier to see the pocket of space in the center of the protein. This pocket, or "active site" is where the oxidation takes place. The actual ADH enzyme consists of 2 identical subunits; only one is shown here.

A little more detail: oxidation of ethanol to acetaldehyde

There are three different molecules that are required for the oxidation of ethanol. They are: 1) the enzyme **alcohol dehydrogenase** or ADH, 2) a zinc atom (Zn), and 3) a coenzyme called nicotinamide adenine dinucleotide (NAD^+). Let's look at the role of each of these components.

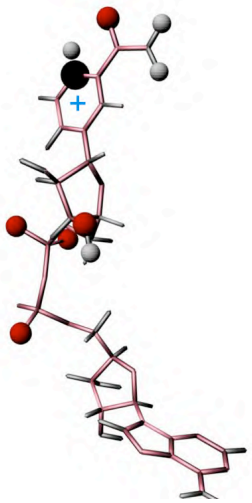
ADH: This enzyme is essential for the oxidation of ethanol to acetaldehyde. Without it, the reaction could take years to happen! There are seven known forms of ADH proteins, each expressed by genes that are named from *ADH1* to *ADH7* (gene names are usually in italics). Each of the forms of ADH facilitates the oxidation of ethanol, although with different rates and efficiencies (see Chapter 4). The enzyme serves as a "place" for ethanol oxidation to occur. It helps to hold all the other components together in the right orientation so that the oxidation can proceed.

Zinc: The Zn atom resides in the active site of the enzyme ADH in its positive-charged form (Zn^{2+}). It is held in place by binding to 3 amino acids exposed in the ADH active site, as well as to a water molecule (these bonds are called "coordinate bonds"). When ethanol enters the active site, the Zn also binds to the oxygen atom of the ethanol molecule, keeping ethanol in the correct orientation long enough for oxidation to take place. Additionally, the Zn is an electrophile, meaning it is electron-loving--it pulls on oxygen's electrons. The Zn serves as a Lewis acid, eventually helping the ethanol lose the terminal H after forming a hydrogen bond with the ADH enzyme.



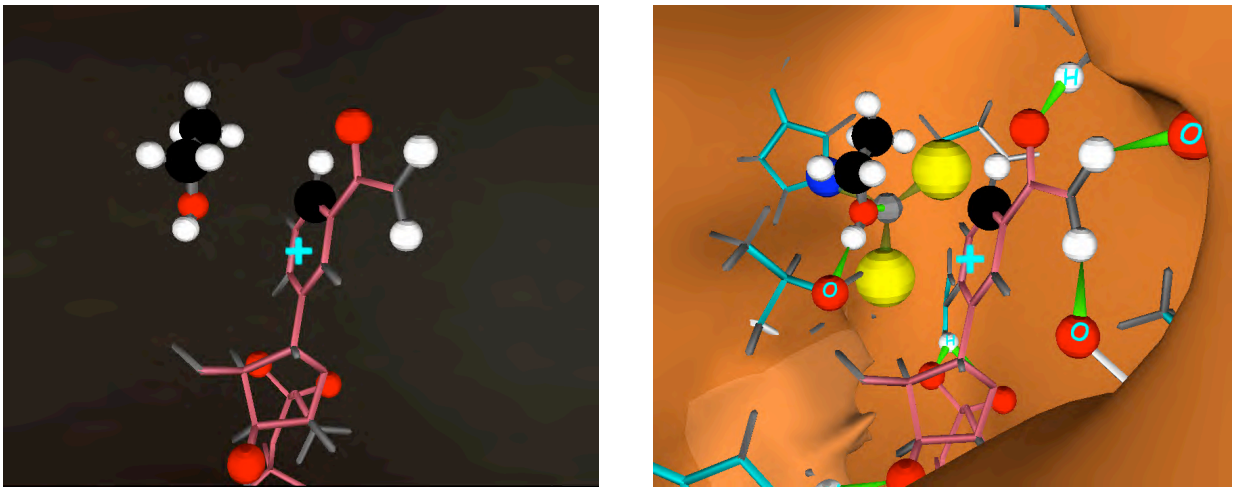
A close-up of the ADH active site reveals the Zn atom (silver) bound to 2 sulfur atoms (yellow) and one nitrogen atom (blue) associated with specific amino acids in the enzyme. This arrangement allows Zn to bind to the ethanol (not shown), effectively tethering it in place.

NAD⁺: This “coenzyme” is what actually performs the oxidation of ethanol, by accepting a pair of electrons (notice that its positive charge attracts the negatively charged electrons from ethanol). NAD⁺, unlike other coenzymes, is not a protein—rather, it is a much smaller molecule. The requirement of NAD⁺ is widespread in biological oxidation reactions.



NAD⁺ is the “coenzyme” that oxidizes ethanol as well as many other biological molecules in our cells. Notice the positive charge on the ring.

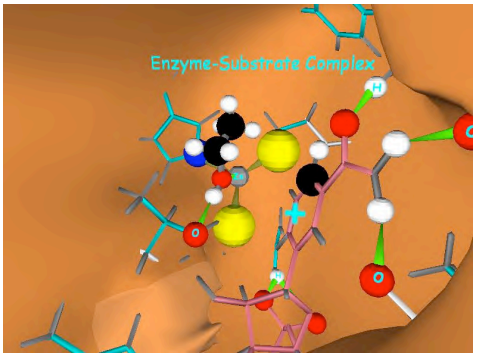
Thus, without ADH and a zinc atom, the probability that ethanol and NAD⁺ could orient themselves to facilitate the transfer of electrons is infinitely small.

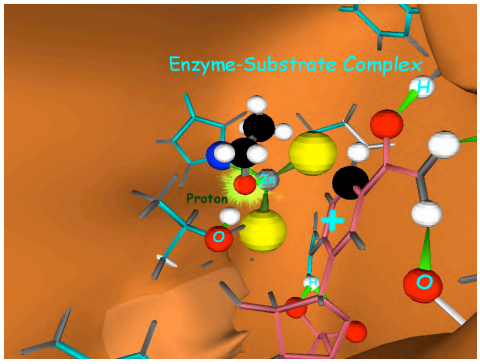


Both panels show that the ethanol and NAD+ are lined up for oxidation reaction. In the left panel, there is no enzyme present, so the freely moving molecules are unlikely to “find” this orientation. In the right panel, the enzyme ADH helps tether the ethanol and NAD+ (in pink) in the right orientation (with hydrogen bonds, in green) for oxidation to proceed. The Zn (silver atom), bound to the ADH, helps hold the ethanol into place too.

Now the steps of ethanol oxidation can be described. NAD⁺ enters the ADH active site in preparation for oxidation; it forms several hydrogen bonds to amino acids lining the active site, holding it in place. (In the pictures, the hydrogen bonds are shown in green.) Ethanol molecules “wander” into the active site by a process known as Brownian motion, the completely random movement of molecules in a liquid. Once in the active site, the ethanol oxygen forms a coordinate bond with the Zn, so that it can be oriented right next to the NAD+ in preparation for the oxidation. Look at the 4 panels below to follow what happens next.

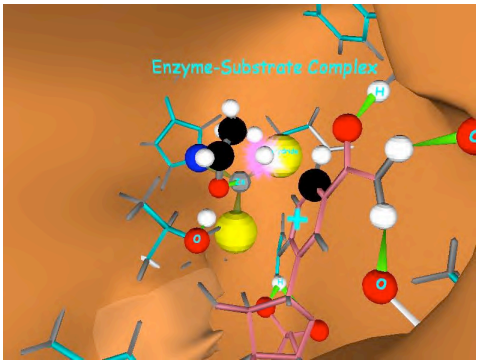
A hydrogen bond forms between the ethanol H (white atom) and the ADH oxygen (red atom)

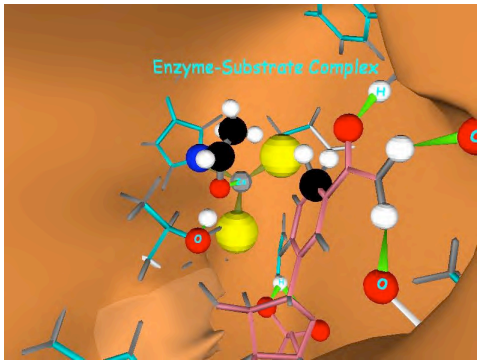




The hydrogen bond breaks, and the proton is transferred to the ADH, leaving an ethanol oxygen with extra electrons (sparkles).

The ethanol loses a hydride (H atom with 2 electrons, in sparkles).



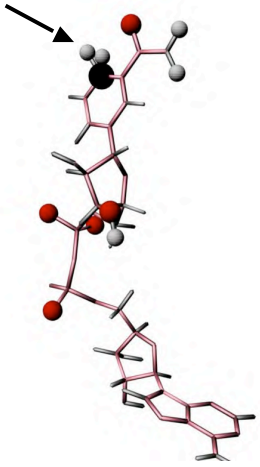


The hydride bonds to the NAD ring, forming NADH.

The 4 panels show the sequence of oxidation.

First, the terminal or hydroxyl hydrogen (O-H) on ethanol forms a hydrogen bond with a specific amino acid in the active site (this is energetically very favorable because of the binding of Zn to the O). This is shown in the upper left panel. Almost immediately, the hydrogen bond between the terminal hydrogen and the amino acid becomes covalent as the amino acid pulls the "naked" proton (H^+, without its electron) off the oxygen, severing the ethanol O-H bond (upper right panel).

This proton transfer leaves the ethanol oxygen negatively charged, with a lone pair of electrons in its outer valence shell (shown in the right panel by sparkles). In this state, it becomes more energetically favorable for the lone electrons to form a double bond with the adjacent carbon, producing what is called a **carbonyl group** (C=O).



NADH, the reduced form of NAD^+ has an additional H on the ring's carbon atom (arrow).

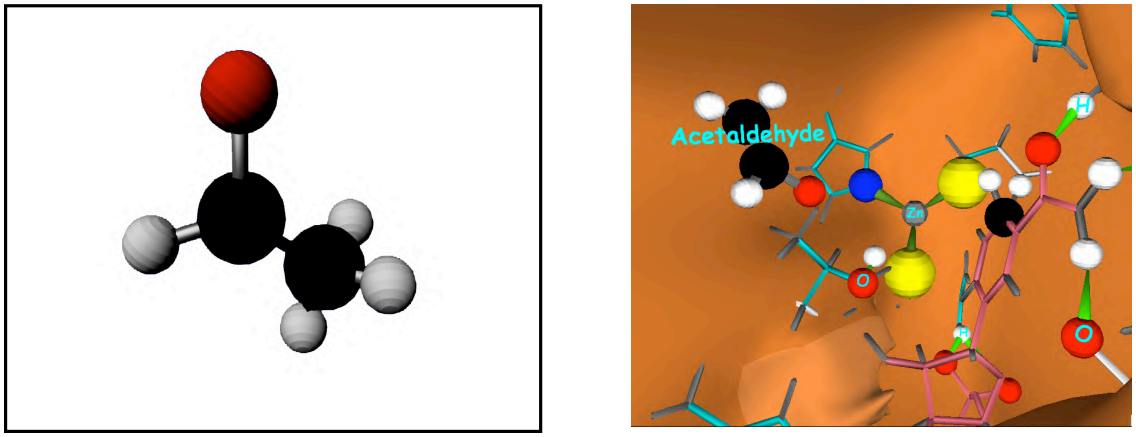
Because this carbon can't violate the octet rule, it must release one of its existing bonds. It does so by losing a **hydride**, which is accepted by the NAD+ (look at the black carbon atom in the bottom right and left panels). A hydride (H^-) is a hydrogen anion, or a proton plus 2 electrons—the hydride is shown with purple sparkles in the lower left panel. **The transfer of the electrons from ethanol to NAD^+ is the actual oxidation step** (bottom right and left panels)**.** At the same time that NAD^+ is reduced to NADH (it gained a H atom, with two electrons), a double bond forms between the carbon and oxygen atoms of ethanol, now forming acetaldehyde. The acetaldehyde is then free to leave the active site.

Thus, NAD^+ is the “oxidizing agent” and becomes reduced in the reaction, while ethanol is the "reducing agent" and becomes oxidized.

So to summarize the steps in oxidation of ethanol, let’s review:

- NAD^+ binds to ADH
- Ethanol binds to ADH and Zn
- Ethanol loses a proton (H+) to ADH
- Ethanol loses a hydride (H^-) to NAD+ (ethanol is oxidized; NAD+ is reduced to NADH)

After NAD^+ is reduced to NADH, it unbinds from ADH and leaves the active site. **The unbinding of NADH is the rate-limiting step in ADH-catalyzed ethanol oxidation**. The faster the NADH comes off the enzyme, the faster the ethanol can be oxidized. The fast metabolism generates a lot of acetaldehyde in a short period of time. As mentioned above, acetaldehyde is a toxic molecule to cells, especially in the liver and gut. Normally it is metabolized by another enzyme (ALDH) to yield an inert product. However, in some people, it is generated too quickly by ADH, or it can even accumulate causing a several unpleasant symptoms (see below and Chapter 4). In people who drink for many years, the buildup of acetaldehyde in cells increases the risk of stomach, colon, and liver cancer.

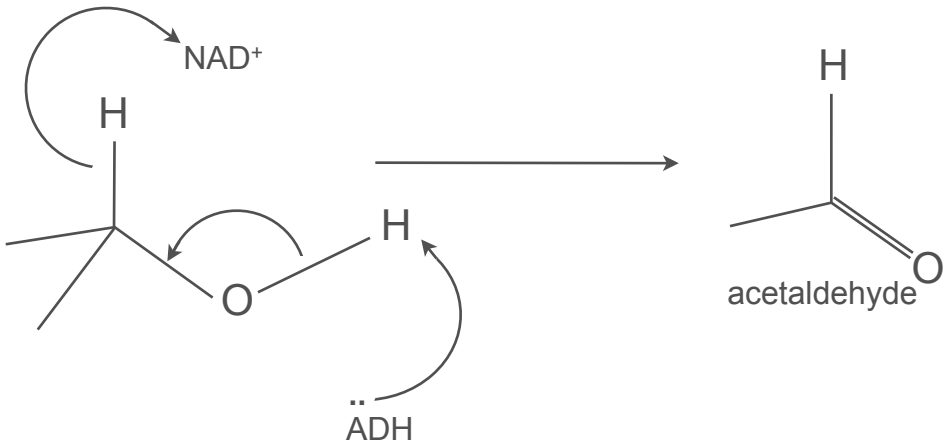


Acetaldehyde (left panel) is generated from the oxidation of ethanol and leaves the active site (right panel). Note that 2 hydrogen atoms are no longer there (1 is removed from the oxygen (red) and the other is removed from the carbon atom). Acetaldehyde is a toxic molecule that makes people feel sick and leads to tissue damage.

There are two last things to consider:

The NADH that is generated can be used later by the cell to perform reduction reactions. During reduction, the opposite process happens: NADH donates a hydride (2 electrons) to a substrate and in turn becomes oxidized back to NAD^+, which is then free to oxidize more ethanol.

Remember the "naked" proton that had been pulled off the hydroxyl group (OH) of ethanol and transferred to ADH (i.e., the first step in the oxidation reaction)? It “moves through” the enzyme and is eventually released into the surrounding cytoplasm. Thus, the enzyme is not actually changed by the reaction. A typical diagram of the oxidation of ethanol to acetaldehyde is shown below:



(Note: in both examples the H^+ was transferred to the ADH itself, and eventually released into the cytoplasm)

The oxidation reaction of ethanol to acetaldehyde can also be written as two “half-reactions” (describing what happens to the donor and the acceptor separately) or a complete reaction that describes the entire reaction in a single equation:

Half reactions:

ethanol $\longrightarrow$ acetaldehyde + H^+ + H^-

H^- + NAD^+ $\longrightarrow$ NADH

Complete reaction:

ethanol + NAD^+ $\longrightarrow$ acetaldehyde + NADH + H^+

The entire oxidation sequence can be viewed online in the interactive virtual reality game that accompanies this tutorial. Go to www.rise.duke.edu/dive-alcohol.

Getting rid of acetaldehyde--oxidation to acetic acid

Since ethanol oxidation produced the toxic metabolite, acetaldehyde, our bodies try to get rid of it. The acetaldehyde is metabolized using another enzyme called **aldehyde dehydrogenase (ALDH)**. Now, the acetaldehyde is oxidized to acetic acid ("vinegar"!), a metabolite used by the cell to produce water and carbon dioxide. The metabolism of the acetaldehyde occurs predominantly inside the mitochondria of hepatocytes (hepatic cells) and also requires NAD^+ to carry out the oxidation. The newly formed acetic acid, along with NADH, then are free to leave the ALDH active site. The process repeats when another pair of NAD^+ and acetaldehyde molecules enters the ALDH active site. For a more detailed discussion about the oxidation of acetaldehyde, see the Appendix.

Enzymes have “behaviors” that matter: kinetics!

The 2 reactions described above can proceed at different rates, either slow or fast. The rate that the enzyme products such as acetaldehyde or acetic acid are formed directly influences what the person

feels after drinking alcohol. Some people feel “high” and others feel sick. Let’s explore how this happens. First we need to understand something about the behavior of enzymes.

Every enzyme has a unique size and shape, as well as preferred substrates, resulting in differences in “behavior” or the way that the enzyme catalyzes a reaction. When speaking of enzymatic “behavior”, we consider the kinetic characteristics, or features related to the ***rate*** of reaction. The two major kinetic features of enzymes include the maximal velocity, represented as the **Vmax**, and the concentration of substrate that produces half the maximal velocity, or the **Km**.

An enzyme’s **Vmax** is a value that reflects the maximum rate, or velocity, at which an enzyme is able to bind to a substrate and catalyze a reaction. A high Vmax indicates a high maximal velocity when the enzymes are at full capacity (meaning there is a lot of substrate present). Another way to think about this is--the Vmax represents the “turnover rate”, or the number of molecules that the enzyme metabolizes in a minute at full capacity.

The other parameter, the **Km**, reflects how tightly the enzyme binds the substrate, but it also includes an indication of the rate of reaction. The Km can be thought of as “efficiency”. For example, an enzyme with a low Km means that the enzyme requires a very small concentration of substrate to achieve half the maximal velocity of the reaction. This is a very efficient enzyme. [Think of an efficient car—it only requires a little gas to go for hundreds of miles.] For more discussion on the parameters of Vmax and Km see the box on the next page.

Kinetics of alcohol metabolism can contribute to ones’ risk of alcoholism

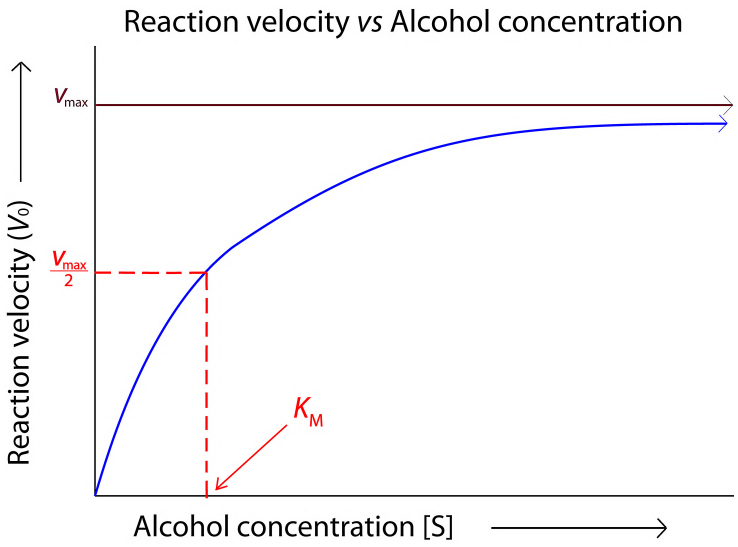
What makes the kinetic properties, Vmax and Km so important? In the case of ADH and ALDH, there are multiple forms (called isoforms) of these proteins expressed by different genes (see Chapter 4). Each isoform of these enzymes has different kinetics. For example, in the case of ADH, some isoforms cause alcohol to be metabolized slowly or inefficiently causing alcohol to accumulate in the blood, leading to intoxication and a high risk of alcoholism. Other isoforms help metabolize alcohol quickly or efficiently, generating the product, acetaldehyde, quickly. This causes a sick feeling that includes a flushed-face, headache, nausea, and rapid heart rate. Furthermore, if one has an ALDH isoform that can’t metabolize acetaldehyde very quickly, then this toxic metabolite accumulates, making matters worse, such as the production of a “hangover” once all the alcohol is gone from the body. With repeated drinking, acetaldehyde can do major damage to body organs such as the liver, stomach, heart, and brain. In addition, acetaldehyde is **carcinogenic**, and is responsible for the high risk of stomach and liver cancer in alcohol abusers.

In Chapter 4, we discuss the genetic basis for expression of different isoforms of ADH and ALDH that differ in their kinetic properties.

A Primer on ADH Enzyme Kinetics

Let's take a closer look at enzyme kinetic properties—the maximal velocity or turnover rate (Vmax) and the efficiency, represented by the Km. We can explore these properties in the context of the action of ADH to catalyze oxidation of alcohol.

We have a constant amount of ADH enzymes in our livers. As alcohol is first ingested, its concentration in the liver begins to increase. Initially, as the concentration of ethanol rises in the liver, the ADH-catalyzed reaction rate increases as well; this means that the number of moles of ethanol oxidized per minute will increase as the ethanol concentration increases. During this time, the oxidation reaction is *first order*--the reaction rate is **dependent** on the ethanol concentration. However, if one drinks more than 1 drink of alcohol (after the first drink), ADH can't keep up with the additional ethanol—that is, the rate of its oxidation falls behind the rate at which ethanol is being delivered to the liver. Thereafter, any increase in ethanol concentration doesn't yield any appreciable increase in rate of metabolism because the ADH enzymes are completely saturated (i.e., at full capacity) and cannot possibly carry out the reaction any faster—the enzyme has reached its Vmax. At this point, the reaction has reached *zeroth order;* the reaction rate is now **independent** of ethanol concentration. Any further increases in the ethanol concentration will have no effect on how fast the ethanol is oxidized. This phenomenon is the basis for the accumulation of alcohol in the blood after drinking more than one drink, causing one to become intoxicated. The kinetic properties of enzyme action can be represented in a typical plot of the rate of oxidation with increasing concentrations of substrate (*e.g.*, alcohol) (see inset).



The Vmax is described using a "turnover number" in units of 1/minute (min^{-1}). The turnover number is a measure of the maximum number of reactions that can be catalyzed by an enzyme in one minute. This number is determined experimentally, when the concentration of the substrate (*i.e.*, ethanol) is very high, thereby saturating all the enzyme active sites. By comparing the Vmax obtained from different isoforms of ADH, one can determine the relative rates of reaction. For instance, the form of ADH expressed by the *ADH7* gene has a turnover number of approximately 1800/min, while the form of ADH expressed by the *ADH1A* gene has a turnover number of approximately 30/min. This means that the former enzyme can catalyze the oxidation reaction about 600 times faster than the latter!

The substrate concentration required for the enzyme to perform at exactly one-half the Vmax is known as the Km—scientists call this the "Michaelis-Menten" constant, named after the scientists who first described the equations for the reaction. A low Km means that a substrate doesn't need to be present in large amounts for the reaction rate to reach one half its Vmax. In addition, the low Km means that the ethanol binds tightly to produce an enzyme-substrate complex that generates the product, acetaldehyde. This is a pretty efficient system. In contrast, a high Km means that a high amount of substrate must be present to even reach one-half the Vmax, which is not very efficient. Let's consider some different isoforms of ADH. For example, the form of ADH expressed by the *ADH5* gene has a Km greater than 1,000 mM, so it is about 33 times less efficient than the form expressed by *ADH7*, which has a Km of about 30 mM. (By the way, the blood alcohol concentration that is defined as legal intoxication (0.08%) is equivalent to about 27 mM ethanol).

CHAPTER 4

Genetic Differences and Alcoholism

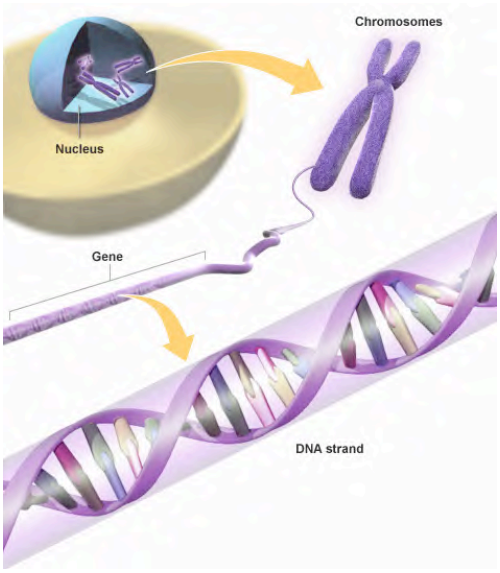
LEARNING OBJECTIVES

Students should be able to:

- *name the macromolecule in which genetic material is stored*
- *describe the structure of this macromolecule*
- *describe the relationship between a genetic polymorphism and an enzyme isoform*
- *explain how genetic differences ultimately result in phenotypic differences among individuals*

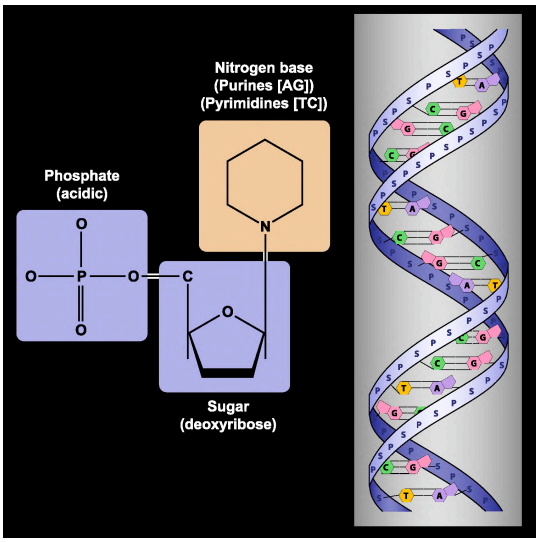
The enzymes involved in ethanol metabolism can have structures that are slightly different in different people. The different enzymes are called isoforms and they are attributed to the differences in genetic makeup (genetic variations) from one person to the next. How is it that slight differences in genes can cause enzymes to function differently in each of us?

Some background: DNA, genes, and the proteins they make



All of our genetic information is stored in macromolecules ("big molecules") known as **deoxyribonucleic acid**, or **DNA**. About half of our DNA comes from the mother, and half from the father. Almost all of our DNA is packaged into structures called **chromosomes**, stored in the nucleus of each cell in our body. Most humans have 46 chromosomes in each cell. A tiny amount of DNA also exists in our mitochondria, an organelle in our cells; mitochondrial DNA is passed to us only through the mother.

Chromosomes consist of twisted strands of DNA (double helix); specific segments of the DNA are called genes.



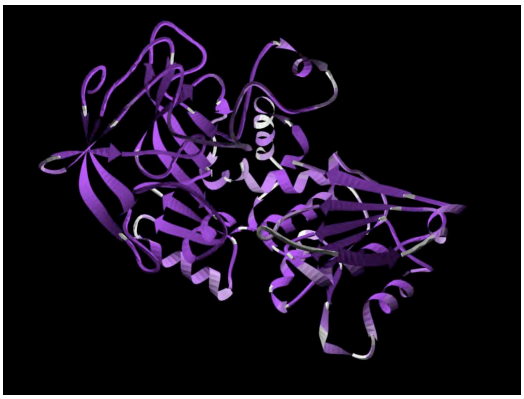
DNA molecules form a double helix. The DNA helix comprises two polymer chains bound together by hydrogen bonds. Each polymer is formed by a string of molecules called **nucleotides**. Nucleotides consist of a nitrogen base, a sugar, and a phosphate. There are four types of nucleotides found in DNA: adenine, thymine, cytosine, and guanine. They are abbreviated as A, T, C, and G, respectively. The way in which these nucleotides are sequenced in the DNA strand dictates all the genetic information of the individual. The two DNA strands are complementary—this means that A always bonds with T, and G always bonds with C. Therefore, if we know the nucleotide sequence of one strand, we know the sequence of the other.

The 2 complementary strands of DNA show the pairing (bonding) of nucleotides—A with T, and C with G. The nucleotides consist of 3 parts, including a nitrogen base, sugar, and phosphate group.

Everything in an individual, from the shape of a nose to an allergy for peanuts, or even to the risk of developing cancer in certain areas of the body, has a genetic basis. Everything about our physical beings is determined by a sequence of 3 billion letters. Here is a sample sequence snippet of the human genome:

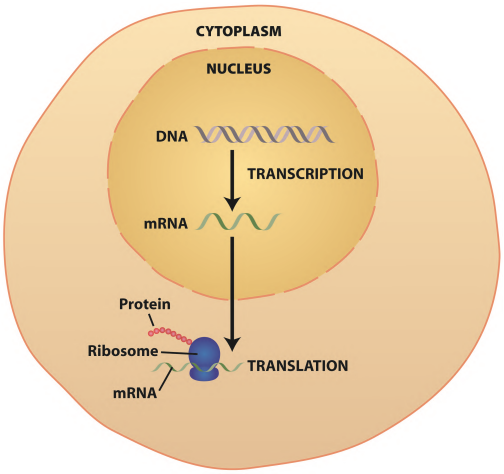
> …TGCATAATATGAGTAAAGTGACAGAAGTCCTAGAGACCAAGTGGGATGCCCGGT
> CCCACAGCCCCAGATCTGGTGCAGCAACCACACCCGGTCTAGCCCACATCACTCC
> CTGAGCAGCACGTCTCCACAGCTTGACAAGGAGGAAGT...

Together, the DNA in each human being is approximately 3 billion nucleotides long. If we were to stretch out the DNA from a single cell, it would span about 3 meters. That's about the height of a typical bedroom, from the floor to the ceiling. If an individual were to link together all the DNA from the trillions of cells within his/her body, it could wrap around the Earth's equator a million times!



The "ribbon diagram" for ADH shows its structure.

DNA is responsible for coding (providing the instructions to synthesize) the entire array of proteins our bodies need in order to function and survive. By some estimates, human DNA contains as many as 30,000 **genes**, which are segments of DNA that code for the proteins. DNA codes for proteins first by **transcribing** its sequence information onto messenger ribonucleic acid, or mRNA, in the cell's nucleus. Then, the mRNA leaves the nucleus and enters the cytoplasm, where ribosomes **translate** the mRNA's information into chains of amino acids (we also get some amino acids from the breakdown of proteins that we eat). These chains of amino acids (called polypeptides) then become functional proteins by "folding" into a specific 3-dimensional shape. Remember the ADH ribbon diagram? This is an example of the folded protein that becomes functional.



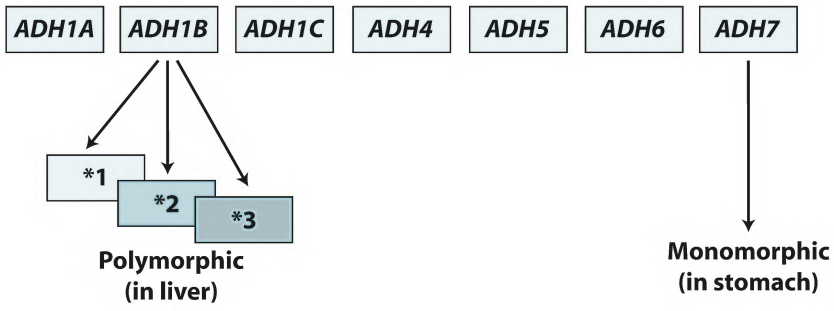
Because every person's DNA sequence is slightly different, with the exception of monozygotic (identical) twins, our bodies' proteins can differ slightly in structure and function. These differences can account for variations in eye color, skin tone, hair curliness, or even personality, and our health. Thus, we are all "special" in our own unique ways.

The steps in protein synthesis are shown. DNA is transcribed to mRNA in the nucleus; the mRNA enters the cytoplasm, where it is translated on ribosomes to form chains of amino acids that result in a protein.

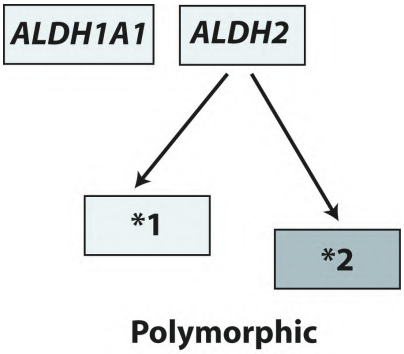
Genes for ADH and ALDH are polymorphic

It turns out that not everyone has the same form of ADH and ALDH. To date, scientists have identified 7 *ADH* genes (gene names are in italics) in humans that code for 7 separate forms of ADH (*i.e.*, **isoforms**) and 2 *ALDH* genes that code for 2 different forms of ALDH. **Genetic polymorphisms**, or natural variations in gene structure, account for this heterogeneity and results in the production of enzymes that are all slightly different in capacity and efficiency (so they have different Vmax and Kms). The combination of specific polymorphisms in our *ADH* and *ALDH* genes (and ultimately, the capacity and efficiency of the enzymes) provide a major basis for the risk of developing alcoholism. (Of course, there are many other genes that play a role as well, and the environment is a very strong factor too).

Note: different forms of an enzyme (protein) are called isoforms, whereas different forms of a gene that makes the protein are called polymorphisms.



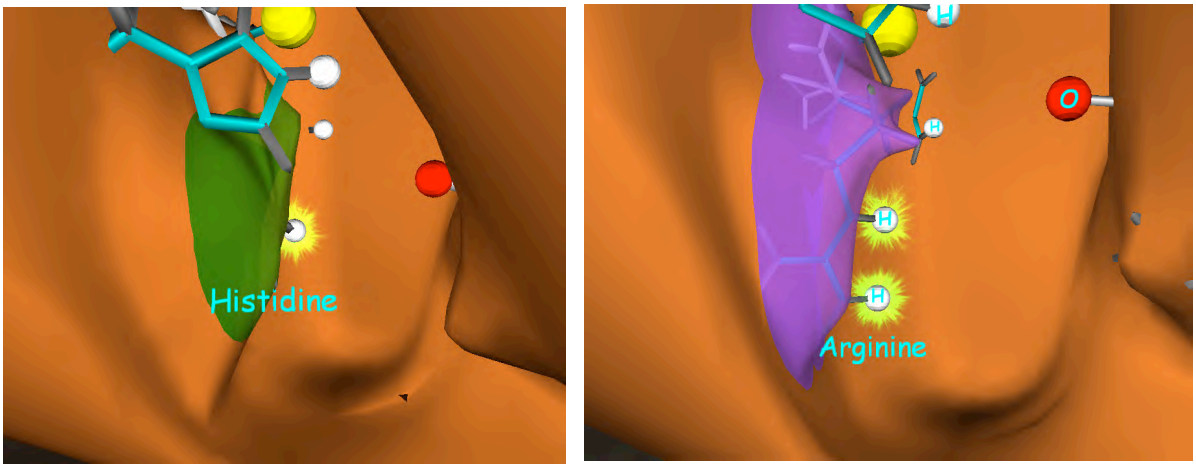
The 7 major genes for *ADH* are shown; the *ADH1B* gene is expressed in 3 different forms.



The 2 major genes for *ALDH* are shown; the *ALDH2* gene is expressed in 2 forms. People with the *ALDH2*2* gene cannot metabolize acetaldehyde—they get very sick!

An example: ADH isoforms and the risk of alcoholism

Let's take a closer look at 2 isoforms of the ADH enzyme. The two forms are encoded by the *ADHB*1* and *ADHB*2* genes—the enzymes differ only by 1 amino acid, otherwise the rest of the 2 proteins are identical to each other. Think of the 2 proteins as 2 strings of 500 pearls (each pearl represents an amino acid). The 48th pearl in the string is green in one case and purple in the other case. The 48th pearl represents the 48th amino acid in the protein and it happens to be lining the active site of ADH. For one form of ADH, the 48th amino acid is histidine, and for the other ADH form, the 48th amino acid is arginine. You can get a better idea of what this means by looking at a picture of the 2 enzymes.

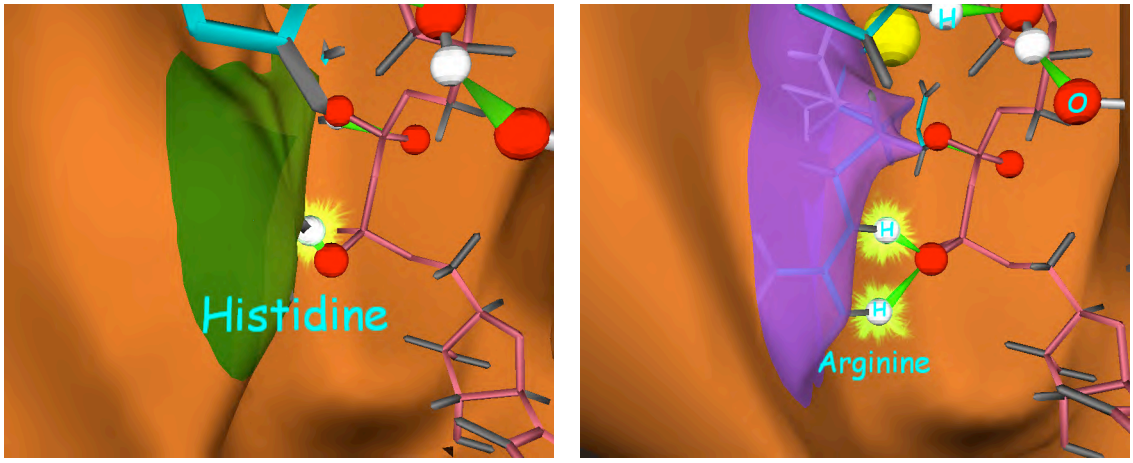


The active sites from 2 isoforms of ADH are shown; they differ by only 1 amino acid (shown in green or purple). Notice the difference in the number of H atoms available (in sparkles) for bonding to NAD^+ (not shown). Histidine has only 1 and arginine has 2.

What is so important about this difference in the 2 amino acids?

If you look at the picture, you'll see that the arginine has 2 H atoms available for bonding to NAD^+, but histidine has only 1 H atom available for bonding. The number of H atoms available is very important in determining how fast the NADH can come off the ADH after oxidation is complete. With 2 H bonds

holding it in place, it's harder for the NADH to come off the enzyme compared to only 1 H bond. So, oxidation using the enzyme with the 2 H bonds showing (arginine) will proceed more slowly compared to the enzyme with only 1 H bond (histidine). The oxidation proceeds more slowly because NAD^+ can't get into the active site if the NADH is still bound there. Remember, the NADH unbinding is the "rate limiting step" in the entire process of ethanol metabolism.



The 2 forms of ADH are shown with the NADH (in pink) still bound. In the presence of the enzyme on the left, the oxidation of ethanol by NAD^+ is fast, and on the right--it's slow.

So what is the result of these slight structural differences? The slower the oxidation proceeds, the more alcohol builds up in the blood and the person is intoxicated more easily. On the other hand, the faster the oxidation, the more acetaldehyde builds up in the blood, and the person gets a sick feeling (flushed face, nausea, dizziness, and rapid heart rate).

The person with the gene that encodes for the slow form of ADH is at increased risk of alcoholism because of repeated intoxication. The person with the gene that encodes for the fast form of ADH is at decreased risk of alcoholism because the sick feeling reduces their amount of alcohol drinking.

The comparison between the 2 forms of ADH can be viewed online in the interactive virtual reality game that accompanies this tutorial. Go to www.rise.duke.edu/dive-alcohol.

Now consider what you've just learned with pictures by applying it to some kinetics. The "fast" enzyme (with only 1 H atom in histidine holding the NADH) is encoded by the *ADHB*2* gene and has a very high Vmax—it can metabolize about 400 molecules of ethanol every minute at full capacity (see table below). The "slow" enzyme is encoded by the *ADH1B*1* gene and has a very low Vmax. It can only metabolize about 9 molecules of ethanol per minute at full capacity. That's about 40 times slower, leading to a quick rise in blood alcohol levels and the onset of intoxication. Check out the table below for some other examples.

Alcoholism and populations

Are you at high risk for alcoholism? To answer this question, you would need to know which *ADH* and *ALDH* genes you carry by sequencing a section of your DNA (or at least you would need some family history). People within the same ethnic population tend to have similar genes and much research has been done on the frequency of several *ADH* and *ALDH* polymorphisms in certain populations. A sampling of current research findings is shown in the tables below. You can see which populations have a prevalence for some of the major *ADH* and *ALDH* polymorphisms, and the behavior (kinetics) of the enzymes encoded by these genes.

ADH polymorphism	ADH Km (μM)	ADH Vmax (turnover # per minute)	Populations with high prevalence
ADH1B*1	0.06	9	Most populations Most Caucasians, Mexican Americans, Native Americans Heavy drinkers
ADH1B*2	0.9	400*	Most Asians, 25% of Jews, some Hispanic
ADH1B*3	34	300*	African-Americans, 6% of Mission Indians (S. West California

ALDH polymorphism	ALDH Km (μM)	ALDH Vmax (turnover # per minute)	Populations with high prevalence
ALDH2*1	1 μM	high	Most populations Alaska Natives (from Asia - interestingly) Pima, Cheyenne, Navajo, Pueblo
ALDH2*2	high ** (very low efficiency)	very low†	40-50% Taiwanese, Han Chinese, Japanese

** this high turnover rate helps to protect against the development of alcoholism—the person generates acetaldehyde quickly and doesn't drink as much due to the sick feeling.

** this low efficiency helps to protect against the development of alcoholism. The person accumulates acetaldehyde and gets sick. Typically, the person tends not to drink alcohol.

** the combination of a very low turnover and efficiency means that the enzyme basically doesn't do anything. These people can't get rid of the acetaldehyde at all—and their risk of alcoholism is very low.

It is important to remember that not all of the populations listed above carry the indicated polymorphisms. Just because most Caucasians have the *ADH1B*1* polymorphism, doesn't mean that *all* do. And, not all Caucasians are predisposed to alcoholism, although the *ADH1B*1* gene is prevalent in heavy drinkers of Caucasian descent.

In terms of the second enzyme, the *ALDH2*2* gene is prevalent in Taiwanese, Japanese, and Han Chinese populations. However, since 40-50% of these populations have the gene, there is a significant portion of the populations without the gene. Without sequencing any individual's DNA, we can't make any assumptions about the genes that they carry. Only their behavior and family history provide a clue.

Understanding Genetics: Misconceptions about Alcoholism in Native Americans

While alcoholism is rampant in many populations across the world, there are misconceptions about the role genetics plays in determining disease rates. It is important to understand that although certain *subsets* of people within *any* population may have genetics that predispose them to alcoholism, there are often other factors that contribute to the alcoholism within a population. For example, Native Americans have the highest rate of alcohol-related deaths of all ethnic groups in the United States—5 to 10 times higher than the general population. What could cause such a difference in alcohol abuse?

There is a common misconception that Native Americans are genetically predisposed to alcoholism, but because the Native American population is very diverse (there are approximately 500 recognized tribes), it is not possible to make such generalizations. Alcoholism in Native Americans did not exist prior to the introduction of the drug by European colonists. A combination of easy availability of alcohol (especially during colonization), destruction of the Native American culture and land, and the forced Western values placed on the Native American society have all contributed to an alcoholism "epidemic" that has persisted throughout generations. However, some subsets of Native American populations do appear to be protected from alcoholism based on their genetics.

The most recent studies regarding variation in *ADH* and *ALDH* genes in Native Americans have focused on a population ("Mission Indians") in the Southwestern area of California. While no individuals expressed the "protective" *ALDH2*2* gene (common in some Asians), about 6% of this population express another gene called *ALDH1A1*2*, which may protect them from developing alcoholism. In addition, the ADH1B*3 allele has been found in about 6% of Mission Indians in Southwest California, who exhibit a third lower alcoholism rates.

The dearth of studies associating alcoholism in Native Americans with genetic variations points to the importance of examining social circumstances in relation with genetic predispositions.

Further reading:

Ehlers, C. (2007). Variations in ADH and ALDH in Southwest California Indians. *Alcohol Research & Health*, 30, p. 14-17.

Szlemko, W., Thurman, P., & Wood, J. (2006). Native Americans and Alcohol: Past, Present, and Future. *Journal of General Psychology*, 133(4), p. 435-451.

Test your knowledge: Tom and Jerry—who is at higher risk for alcoholism?

Can you tell which of these two friends has a higher risk for developing alcoholism based on their expression of ADH and ALDH?*.

*note: there are many other factors (environmental and genetic) that contribute to the risk of developing alcoholism; these are not considered for this exercise

Tom has an *ADH* gene coding for the ADH enzyme that has a very low maximal rate of ethanol oxidation (i.e., a low Vmax). He also has an *ALDH* gene coding for the enzyme that has a very high maximal rate of acetaldehyde oxidation (high Vmax).

Jerry has just the opposite genomic makeup. He as an *ADH* gene that codes for the ADH enzyme with a high maximal rate of ethanol oxidation (high Vmax). He also has an *ALDH* gene that codes for the ALDH enzyme with a low maximal rate of acetaldehyde oxidation (low Vmax).

Who is more prone to alcoholism (the term used today is alcohol use disorder)? Note that the genetic profile merely predicts the risk of having an alcohol use disorder if the person drinks alcohol; it does not predict that the person ***will*** definitely have an alcohol use disorder.

Can you predict the risk to alcoholism someone else has a different combination of ADH and ALDH versions than indicated for Tom and Jerry?

Answer:

Tom is more prone to an alcohol use disorder. Low-capacity ADH enzymes cause the oxidation of ethanol to acetaldehyde to occur slowly—another way of describing the enzyme behavior is that “the turnover rate” is slow. Fewer molecules of ADH are oxidized per minute. Because ethanol in moderate amounts is mostly associated with positive feelings in the brain, and acetaldehyde is mostly associated with negative feelings (and also with tissue damage, see Chapter 3), Tom is more likely to "enjoy" the alcohol. Moreover, because he has efficient ALDH enzymes, the acetaldehyde that *is* produced is quickly oxidized to acetic acid, minimizing any of its unpleasant effects. The net effect is that soon after drinking, the concentration of ethanol in Tom’s blood (and brain) is far greater than that of acetaldehyde; he feels much of the rewarding effects and little of the negative effects.

On the other hand Jerry, with a high capacity ADH generates acetaldehyde quickly, providing quick appearance of some of the negative effects associated with alcohol use (i.e., flushed face, nausea, dizziness, headache). To make matters worse, Jerry’s ALDH has a very low capacity—it saturates quickly and works slowly, causing accumulation of acetaldehyde in Jerry’s bloodstream. The negative effects are prolonged, and in most cases, people like Jerry don’t like to drink alcohol. So his genetic profile predicts that he will not develop an alcohol use disorder.

The two other combinations of ADH and ALDH not listed above can lead to some alcoholism risk, but probably not as great as Tom’s risk.

Glossary

Acetaldehyde – an organic compound with the formula CH_3CHO. This toxic compound is produced when ethanol is oxidized and it can cause increased heart rate, headache, nausea, and flushed face in some people. It is also carcinogenic.

Acetic acid – (vinegar) a metabolite that is produced when acetaldehyde is oxidized. It is a weak, monoprotic acid that is used by cells in general metabolic reactions.

Active site – the part of the enzyme into which the substrate binds to participate in the catalytic reaction.

Alcohol dehydrogenase (ADH) – an enzyme that catalyzes the oxidation of ethanol (as well as other alcohols), yielding acetaldehyde.

Aldehyde dehydrogenase (ALDH) – an enzyme that catalyzes the oxidation of acetaldehyde, yielding acetic acid.

Anaerobic – indicates a condition "without air," or without oxygen. The opposite of anaerobic is aerobic.

Blood Alcohol Concentration (BAC) – the amount of ethanol in grams dissolved in a deciliter (100 milliliters) of blood and expressed as a percent. In the United States, if a person has a BAC of 0.08%, (s)he is considered to be legally intoxicated.

Brownian motion – the random movement of molecules in a liquid or gas due to thermal energy.

Capillaries – tiny vessels that carry blood from the arteries to the veins. The capillaries deliver oxygen and nutrients to every cell in the body; their permeable walls allow drugs to pass through easily as well.

Carbonyl group – a group of 2 atoms (a carbon and oxygen) bound together with a double bond (C=O). The oxygen is often referred to as the carbonyl oxygen while the carbon is referred to as a carbonyl carbon.

Catalyze – to speed up the rate of a chemical reaction by a substance that is not consumed or changed by the reaction. The substance, usually an enzyme, provides a reaction pathway that requires a lower activation energy.

Chromosomes – long strands of DNA, coiled around specific proteins, that contain the genetic information for an organism. Humans have a total of 46 (23 pairs) chromosomes that reside in the nucleus of cells.

Coenzyme – a small organic molecule that facilitates the catalytic function of certain enzymes. Often, a coenzyme's structure changes during a reaction but it eventually returns to its original form through

subsequent reactions.

Concentration gradient – a difference in the amount of chemical substance per unit volume usually on different sides of a barrier or membrane.

Deoxyribonucleic Acid (DNA) – a large molecule in the cell nucleus that provides the instructional code for the synthesis of proteins. DNA consists of two complementary polynucleotide chains coiled to form a double helix.

Distillation – to separate or purify a substance through evaporation and then condensation.

Enzyme – a protein that catalyzes a chemical reaction by facilitating the rate at which the reaction occurs. It binds to one of the reactants, a substrate, to facilitate a change in its structure, forming a different molecule, or product. Endothelial cell – a cell that has no contact with the external environment (*e.g.*, cells lining the blood vessels, liver cells).

Epithelial cell – a cell that has a direct connection to the external environment (*e.g.*, skin cells, cells lining the stomach, nose,or bladder).

Ethanol – also known as alcohol. An organic compound containing two carbons with the following structural formula: C_2H_5OH.

Equilibrium – a state in which the ratio of the concentrations of a compound in two compartments (*e.g.*, tissue and capillary) achieves a constant value while the reaction is still occurring. There is no net movement of the compound between the compartments.

Fermentation – a process by which yeast metabolizes sugar to yield ethanol and carbon dioxide. This process is necessary for energy production in anaerobes.

Filtration – diffusion of molecules through pores or water spaces across a membrane; it is limited by the size of the molecule and occurs in the direction of the concentration gradient. No energy is required.

Gastrointestinal (GI) tract – a group of organs (esophagus, stomach, small intestine, and large intestine) connected to each other; they carry out food ingestion, digestion, and defecation.

Genes – segments of DNA that code for hereditary characteristics; they are present within the chromosomes.

Genetic polymorphism - multiple forms of a given gene (small nucleotide sequence differences) that exist within a population.

Hepatocyte – the major cell type found in the liver; they are rich in metabolizing enzymes.

Hydride – a hydrogen atom that has 2 electrons associated with it.

Hydrophilic – “water-loving”; indicates a compound or substance that dissolves readily in water. Charged compounds are hydrophilic.

Hydrophobic – “water-fearing”; indicates a compound or substance that is soluble in fat, or in organic solvents. Compounds with chains of carbon atoms are hydrophobic.

Km – a value that reflects how tightly the enzyme can bind to a particular substrate. It is expressed as the concentration of substrate binding to an enzyme that allows the reaction to proceed at half the enzyme’s maximal velocity (Vmax).

Metabolism - the chemical processing of a compound to yield energy or products for use by the body,

Metabolize – to chemically process a compound to yield energy or products for use by the body, or for elimination from the body.

Mitochondria – organelles found in the cytoplasm of every eukaryotic cell; many enzyme reactions occur here, as does cellular respiration. They are the power generators of the cell.

Nicotinamide Adenine Dinucleotide (NAD+) – a coenzyme found in living cells that helps transfer electrons during metabolic reactions (*e.g.*, oxidation).

Nucleotides – chemical compounds that consist of a nitrogenous base, a sugar, and a phosphate group(s). Nucleotides are the structural units of DNA and RNA.

Oxidation – the donation of electrons to another atom, often by removal of a hydrogen (H atom); there is an increase in oxidation number.

Oxidation state – the theoretical charge a molecule would have if all the bonds in the molecule were 100% ionic. For simple atoms of ions, the oxidation state is equal to the atom or ion's ionic charge. The higher the oxidation state, the greater the degree of oxidation. Also referred to as the oxidation number.

Oxidizing agent (oxidant) – an element or chemical compound that readily transfers oxygen atoms or a substance that gains electrons in a redox reaction.

Passive diffusion – the movement of non-polar molecules across a biological membrane. Movement occurs with the concentration gradient and no energy is required.

Portal vein – a large vein that carries blood from the digestive tract to the liver.

Proton transfer - the process by which a hydrogen atom (minus its electrons) moves from one compound to another.

Pyloric sphincter – a small muscle that opens and closes a passageway between the stomach and small intestine . The pyloric sphincter controls the movement of stomach contents into the small intestine.

Redox reaction – an oxidation-reduction reaction; reflects the simultaneous reactions of oxidation and reduction. Redox reactions involve a change in the oxidation number of the reactants and the products.

Reducing agent (reductant) – an element or compound that can donate electrons to another substance in a redox reaction or decrease the oxidation number in another substance.

Reduction – the gain of electrons from another atom, often by addition of a hydride (a hydrogen atom with its 2 electrons).

Substrate – the reactant in an enzyme-catalyzed reaction. The substrate binds to the active site within the enzyme.

Total body water – the water volume of the body, including intracellular and extracellular water. On average, males have more water volume as a percentage of their body mass than females.

Transcribe – (transcription) the process in which genetic information contained in DNA is converted to a more accessible form called messenger RNA (mRNA). The process is directed and regulated by several enzymes within the cell nucleus.

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

Vmax – a value that reflects the maximum rate at which an enzyme is able to bind to a substrate and catalyze a reaction. Units are usually expressed as # molecules/min and reflects “turnover” (number of molecules metabolized per minute)

Teacher's Guide to "Drunk Flies and Genetics"

Background information
At a molecular level, this activity demonstrates how a gene controls the production of an enzyme, whose function can be measured by observing a behavior.

Student responsibilities
The laboratory part of this activity requires that the students have a basic understanding of alcohol and its effects on the body, protein synthesis, and Mendelian genetics. Moreover, the students will need to watch their flies over a 24-hour period; you may wish to require them to take the flies home for a night to observe their behavior and record the data. If not, ask them to stop by the classroom before and after school to collect observation data at different time points.

Class time needed
The activity can take up to two class periods, depending on how much time the instructor allows for student exploration. The actual laboratory activity takes 20 minutes. A period of 20 minutes the following day gives ample time to discuss class data.

Abstract of activity
If you open any magazine or newspaper these days, you can typically find an article on the most recent genetic breakthrough. In the past, many of these discoveries have been about a genetic disease, but now, more and more studies are implicating gene mutations in the risk of many diseases such as cancer, heart disease, mental illnesses, and drug addiction. For example, gene variations have been found to 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* (common fruit fly) is an excellent model for behavioral effects of alcohol, based on the work of Ulrike Heberlein** at the University of California at San Francisco. Like people, flies possess the ADH (alcohol dehydrogenase) gene, which controls the production of the alcohol dehydrogenase (ADH) enzyme. Flies possessing the dominant ADH gene have the ability to oxidize ethanol to acetaldehyde:

$$NAD^+ + ADH + alcohol \text{ --------> } NADH + aldehyde \text{ (or ketone)} + H^+$$

Some people have variations in their ADH genes that affect their ability to metabolize alcohol. So scientists have genetically-engineered “ADH minus” flies--flies with a mutation in the ADH gene, rendering it non-functional. Without a functional ADH gene or enzyme, the flies cannot break down ethanol, leading to visible inebriation in their behavior . Ultimately, they can die of alcohol poisoning due to their inability to metabolize (and get rid of) it.

In this activity, students develop and run a laboratory comparing alcohol-induced intoxication in normal (“wild-type”) flies and ADH- flies with the ADH gene mutation.

Note: Before starting the activity it might be useful to review the basics of biological or chemical reactions and enzyme function using the following questions.

1. Why are enzymes so important to make biological reactions go?
2. What happens to the reaction if there is little or no enzyme present?
3. What other kinds of diseases (other than alcoholism) are the result of enzyme deficiencies in the body?

Materials

- **Fruit flies (*Drosophila)***
  Flies can be obtained from biological supply houses. For example, two cultures of ADH- and ADH+ fruit flies are available from Carolina Biological, #171970 or as part of the entire kit #171969. They *may* also be available free of charge from the Mid-American Drosophila Stock Center. Call the Curator of Stocks (419-372-2631) for wild type (normal) and ADH-negative flies (strain 1383F1 ADH -fn23 cn bwD). As only one vial of each type of flies is sent, place your order two months prior to the activity date. It takes about one week for the flies to hatch upon arrival, and two weeks for each subculture to increase the fly population to reach the size necessary for classroom use.

- **Fly vials**
  Enough for subculturing the fly strains and at least one vial and sponge for every lab pair. The vials can be ordered separately or as part of a kit (e.g., Carolina Biological).

- **Alcohol**
  White wine (~10 ml per group)

  Note: *This lesson requires the use of white wine as the source of alcohol for the fruit flies. Before proceeding, check your school or school district policy regarding alcohol on school premises. You may have to request special permission. If the use of white wine is not allowed, the activity can also be performed with rubbing alcohol (isopropyl alcohol) or 95% ethanol found in laboratories.* ***Do not*** *use red wine, since observers will not be “blind” to the treatment condition. See below.*

- **Other**
  Instant fly media, cotton balls, stirring rods, pipettes, small graduated cylinders, white wine (or rubbing alcohol) (note previous caution about alcohol in schools), delicate paint brushes, refrigerator, ice bucket.

Procedure

A. Presort about 20 flies into several vials (1 per group) before the lab. These flies can be anesthetized when class starts by chilling them in a refrigerator for 5-10 minutes. Keeping them on ice afterwards also slows their revival. Directions for care and maintenance of Drosophila are available at http://biology.arizona.edu/sciconn/lessons2/Geiger/intro2.htm.

B. Start off with a discussion about alcoholism and its effects on the body and on society. As the discussion of the causes of alcoholism ensues, revisit the idea that differences in genetics can underlie differences in phenotype (what is observed). The students should be familiar with this concept after using the *DiVE into Alcohol* program. Introduce the idea that fruit flies can be used as a model of the human situation in which there are differences in the ability to metabolize alcohol (based on the gene for ADH), leading to differences in the behavioral effects of alcohol. (Also consult the *DiVE into Alcohol* tutorial and web-based virtual-reality program at www.rise.duke.edu/dive-alcohol)

C. Ask students: "How would you test for alcohol intoxication in fruit flies?" Guide the students to design a procedure to answer the question. Compare their solutions with the following protocol that works quite well (and is fun). Then have students work through the steps of this protocol.

1. Each person should obtain an empty vial, 3-4 small cotton balls, and a stirring rod.
2. Each person should wedge the cotton balls in the bottom of their vial.
3. Working in teams of four, each student chooses an experimental condition to perform:

   Person A: ADH- flies, water cotton Person C: ADH- flies, alcohol cotton
   Person B: ADH+ flies, water cotton Person D: ADH+ flies, alcohol cotton

   The water serves as a control for the experiment.

4. Persons A and B will add 5 ml of water to their vial. Persons C and D will add 5 ml of wine (or alcohol) to their vial. Each cotton ball should be soaked but not submerged. Using the stirring rod, tap down the cotton balls to wedge them in place and to drain off excess fluid. 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 become wet. (Flies can drown in drops of fluid)

6. Use a piece of tape and a marker to label the vial with the student's name, type of flies and whether water or alcohol is included. **(Note: later on you will remove the labels and code the vials so that the students are "blind" to the treatment condition)**

7. Provide each group a vial of chilled flies. The flies in the vial should not be moving. If the flies wings or legs appear to trembling they should be chilled further. As soon as the flies warm up, they wake up and fly away.

8. Open the chilled vial and pour the flies onto a piece of paper. Using the paintbrush, gently sweep 5 flies of the strain to put into each of the 4 labeled vials. Stopper the vial, but leave the vial on its side so the "sleeping" flies will not drown in the wet cotton.

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

10. The teacher should replace the tape on each vial with a new piece of tape with a code: A, B, C, D. 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).

11. Observe the flies over the next 24-hours at the approximate times listed below. Students make a data table of their observations for vials A,B, C, and D. Note all behaviors observed. **(Note: it’s best that the students make the data table for observations BEFORE they start the experiment….the table should be printed out and ready for data collection which will start 15 minutes after loading the flies into the vials).**

    Collect observation data at:
    15 minutes
    30 minutes
    60 minutes
    4 hours
    8 hours
    Next morning (students should decide on a time, or record the time (in their table) that they actually performed the observation)

    One way to turn qualitatitive data into quantitative data is to have the students collect the data as frequency or percentage data at each time point. For example, construct several behavioral endpoints to measure and assign a percentage of the flies exhibiting the behavior as shown here:

Behavior	# showing behavior	% showing behavior
Flying around	3	60%
Some movement	1	20%
No movement (but alive)	0	0%
Dead	1	20%
Total flies in vial	5	100%

12. After all data are collected and compiled (24 hours later), ask the students if they can predict which condition (ADH positive or minus, water or alcohol) was in their vial. Then break the code and tell each student what condition they actually tested. Determine how many students figured out their condition correctly.

13. Have a discussion 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.)

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

15. Tell students they will be required to turn in a formal laboratory report on the fly activity including an introductory statement, procedure, data tables and conclusion. (The teacher should make a Lab Report Rubric ahead of time to give to the students).

Note:

This activity was adapted from: Virginia Commonwealth University/Life Sciences Secrets of the Sequence classroom lessons)
http://www.pubinfo.vcu.edu/secretsofthesequence/lessons/sots_lesson_101_2.pdf

The activity was developed originally by teachers in a Rutgers State University and National Association of Biology Teachers summer program. See link to Theresa Peters at http://www.accessexcellence.org/AE/AEC/AEF/1996/peters_tolerance.php

Assessment

Chapter 1

1. Ethanol is a molecule that has both a hydroxyl group (-OH) and a short, hydrocarbon chain (-CH2-CH3). If the hydrocarbon chain were longer, how would the solubility of this new alcohol in water change?

   A. The alcohol would be more water soluble because the longer hydrocarbon chain would provide more non-polarity to the molecule
   B. The alcohol would be less water soluble because the longer hydrocarbon chain would provide more non-polarity to the molecule
   C. The alcohol would be more water soluble because the longer hydrocarbon chain would provide more polarity to the molecule
   D. The alcohol would be less water soluble because the longer hydrocarbon chain would provide more polarity to the molecule

2. Which of the following describes the passive diffusion of ethanol from the gut into the bloodstream?

   A. The diffusion requires pressure within the GI tract
   B. The diffusion requires energy
   C. The diffusion involves movement from low concentration to high concentration
   D. The diffusion involves movement from high concentration to low concentration

3. Which of the following describes why food would delay ethanol absorption into the bloodstream?

   A. The pyloric sphincter closes in the presence of food, preventing ethanol from entering the small intestine
   B. The food competes with the ethanol to be absorbed through the membranes of the GI tract
   C. The food causes release of acids in the stomach, which destroy the ethanol
   D. The food activates release of stomach hormones, which destroy the ethanol

4. The enzyme, alcohol dehydrogenase (ADH), facilitates a chemical reaction to degrade alcohol. It is present in the stomach of males but not females. If both the male and female drink a glass of wine (same amount), how will their blood levels of alcohol differ?

   A. The male will have a higher blood alcohol level than the female
   B. The female will have a higher blood alcohol level than the male
   C. The male will have the same blood alcohol level as the female
   D. Initially, the blood alcohol in the male will be lower than the female's, but by the end of the glass, the amount of alcohol in their blood will be the same

5. Which features distinguish epithelial cells from endothelial cells?

   A. Epithelial cells are very permeable compared to endothelial cells to substances
   B. Epithelial cells have access to the external environment; endothelial cells do not
   C. Endothelial cells are not very permeable compared to epithelial cells to substances
   D. Endothelial cells have access to the external environment; epithelial cells do not

Chapter 2

1. The capillary cell membranes are fairly leaky—they have many pores and the cells are loosely packed. How does this structure enable ethanol to get into the capillaries so easily?

   A. The pores in the membrane and water spaces between cells allow ethanol to passively diffuse through them due to its polar character
   B. The pores in the membrane and water spaces between cells allow ethanol to be actively transported through them due to its small size
   C. The pores in the membrane and water spaces between cells allow ethanol to filter through them due to its small size
   D. The pores in the membrane and water spaces between cells allow ethanol to filter through them due to its polar character

2. The ethanol that is not metabolized in the liver enters the venous system and continues to circulate through the bloodstream. Which describes the path that the ethanol takes once it enters the venous system?

   A. → lungs → right side of the heart → left side of the heart → all body areas
   B. → left side of the heart → lungs → right side of the heart → all body areas
   C. → lungs → left side of the heart → right side of the heart → all body areas
   D. → right side of the heart → lungs → left side of the heart → all body areas

3. Like most chemical systems, the ethanol in the bloodstream eventually reaches an equilibrium with the ethanol in the tissues. How is equilibrium best defined?

A. The concentration of ethanol in the bloodstream equals the concentration of ethanol in the tissues
B. The ethanol molecules no longer move back and forth between the bloodstream and the tissues
C. The net movement of ethanol molecules between the bloodstream and the tissues becomes zero
D. The rate of movement of ethanol molecules into bloodstream and tissues is the same

4. Relative to females, males have a lower percentage of their body mass as fat. If a male and female drank equivalent amounts of alcohol, who would have a higher concentration of ethanol in their bloodstream?

A. The female because she has less water spaces in her body
B. The female because she has more water spaces in her body
C. The male because he has less water spaces in his body
D. The male because he has more water spaces in his body

Chapter 3

1. NAD+ plays an important role in biochemical reactions. What role does it play in the metabolism of a molecule like ethanol?

A. NAD+ reduces ethanol by accepting a pair of electrons
B. NAD+ reduces ethanol by donating a pair of electrons
C. NAD+ oxidizes ethanol by accepting a pair of electrons
D. NAD+ oxidizes ethanol by donating a pair of electrons

2. Substrates bind to enzymes with a “lock-and-key” fit. Why is this arrangement important?

A. It ensures that the enzymes are consumed in the reaction
B. It ensures that the substrate can’t be released from the enzyme during the reaction
C. It ensures that the substrate binds to the enzyme with an orientation that allows the reaction to occur
D. It ensures that substrate is completely metabolized in the reaction

3. If an enzyme binds more tightly to substrate X compared to substrate Y, then which of the following is true?

A. The enzyme has a lower Km for substrate X than for substrate Y; it is more efficient

B. The enzyme has a higher Km for substrate X than for substrate Y; it is more efficient
C. The enzyme has a lower Vmax for substrate X than for substrate Y; it is more efficient
D. The enzyme has a higher Vmax for substrate X than for substrate Y; it is more efficient

4. What does the enzyme aldehyde dehydrogenase (ALDH) do?

A. It catalyzes the oxidation of ethanol into acetaldehyde
B. It catalyzes the oxidation of acetaldehyde into acetic acid
C. It catalyzes the reduction of ethanol into acetaldehyde
D. It catalyzes the reduction of acetaldehyde into acetic acid

Chapter 4

1. Which best describes protein synthesis?

A. It is governed by DNA, but messengers (mRNA) help in the process
B. It begins with translation and then proceeds with transcription
C. It is carried out in the cell nucleus
D. It is carried out by amino acids

2. Part of a DNA strand reads AATCGTGC. Its complementary strand is:

A. TTCGATGG
B. TTAGCACG
C. AACTAGGA
D. TAGCTAGC

3. In relation to alcoholism, how do genetic differences influence phenotype (i.e., observable) differences among individuals?

A. Genetic differences only increase an individual's susceptibility to alcoholism
B. Genetic differences only decrease an individual's susceptibility to alcoholism
C. Genetic differences may increase or decrease an individual's susceptibility to alcoholism
D. Genetic differences cannot influence an individual’s susceptibility to alcoholism

Open-Ended Questions

These comprehensive questions are intended to test student understanding of the material:

1. Describe the distribution pathway of ethanol once it enters the bloodstream. Where does it go and how do people end up getting “drunk”?

2. NAD+ plays an important role in the oxidation of ethanol to acetaldehyde. Discuss the role of NAD+ in this reaction and what happens to its oxidation state. Then predict the role of FAD+ in a similar biochemical reaction.

3. Bonds always form and break during chemical reactions. Describe at least one example of when hydrogen and covalent bonds form or break during the metabolism of ethanol. Which type of bond is stronger? Why?

4. Mark has a genetic mutation that causes his ADH enzyme to catalyze his metabolic process much *slower* than most males. What does this mean in terms of his blood alcohol levels and in terms of how alcohol affects his body?

Elements of Understanding

Students who construct a sophisticated understanding of ethanol metabolism and of the general principles of solubility, oxidation, and enzyme kinetics should be able to:

- Rank order the solubility in water of alcohols with increasing numbers of carbon atoms
- Indicate the relative polarity of different chain-length alcohols
- Identify chemical compounds that are oxidizable
- Rank order bond strength among different types of bonding forces
- Define the difference between oxidation and reduction
- Describe the role of NAD+ and NADH in biochemical reactions
- Predict the role of FAD+ and FADH in biochemical reactions
- Predict how changes in enzyme kinetics (Km and Vmax) affect the rate and extent of product formation

Resources

Chapter 1

Books and Articles

Meyer, J. & Quenzer, L. (2005). *Psychopharmacology: Drugs, the Brain and Behavior*. Sunderland, Massachusetts: Sinauer Associates, Inc. Chapter 9: Alcohol.

Paper on differences in alcohol metabolism:

Lieber, C. (2000). Ethnic and gender differences in ethanol metabolism. *Alcoholism: Clinical And Experimental Research*, 24(4), p. 417.

Paper on the gastric activity of ADH:

Seitz, HK., Egerer, G., Simanowski, UA., Waldherr, R., Eckey, R., Agarwal, DP., Goedde, HW., & Von Wartburg, JP. (1993). Human gastric alcohol dehydrogenase activity: effect of age, sex, and alcoholism. *Gut Online*, 34(10), p. 1433-1437.

Paper on how carbonation may affect absorption:

Roberts, C. & Robinson, SP. (2007). Alcohol concentration and carbonation of drinks: the effect on alcohol blood levels. *Journal of Forensic and Legal Medicine,*14(7), p. 398-405.

Web-based References

Background on how alcohol was discovered:
"Alcohol Consumption." Encyclopedia Britannica Online. 2008. http://search.eb.com/eb/article-251723

The Anatomy of the Gastrointestinal Tract and Drug Absorption:
http://www.chemcases.com/pheno/pheno14.htm

Alcohol Pharmacology Education Partnership: A Curriculum for High School Biology & Chemistry
http://www.rise.duke.edu/apep

Chapter 2

Books and Articles

Meyer, J. & Quenzer, L. (2005). *Psychopharmacology: Drugs, the Brain and Behavior*. Sunderland, Massachusetts: Sinauer Associates, Inc. Chapter 9: Alcohol.

Web-based References

Alcohol, Chemistry, and You:
http://www.chemcases.com/alcohol/alc-05.htm

Information on Capillaries:
http://www.fi.edu/learn/heart/vessels/capillaries.html

Alcohol Pharmacology Education Partnership: A Curriculum for High School Biology & Chemistry
http://www.rise.duke.edu/apep

Chapter 3

Books and Articles

Chang, R. (2003). *General Chemistry: The Essential Concepts, 3rd Edition*. New York: McGraw Hill. Chapter 4: Reactions in Aqueous Solutions.

Loudon, G.M. (2002). *Organic Chemistry, 4th Edition*. New York & Oxford: Oxford University Press. Chapter 10: Chemistry of Alcohols and Thiols.

Paper on acetylaldehyde dehydrogenase (ALDH) oxidation mechanism:

Fridovich, I. (1966). The mechanism of the enzymatic oxidation of aldehydes. *Journal of Biological Chemistry*. 214, (13); p. 3126-3128.

Web-based References

Oxidation Videos:
http://www.youtube.com/watch?v=a6RR4kPsnlE (oxidation - reduction reaction)
http://www.youtube.com/watch?v=BhxdxBX4hZs (oxidation in our bodies)

http://www.youtube.com/watch?v=1oVJ5E8kdWs (oxidation of copper)
http://www.youtube.com/watch?v=gmvW_5I3m_U (oxidation of alcohol)
Alcohol Pharmacology Education Partnership: A Curriculum for High School Biology & Chemistry
http://www.rise.duke.edu/apep

Chapter 4

Books and Articles

Paper on Native American use of alcohol:
Szlemko, W., Thurman, P., & Wood, J. (2006). Native Americans and alcohol: past, present, and future. *Journal of General Psychology, 133*(4), p.435-451.

Papers on genetics and alcohol:

Birley, A. J., James, M. R., Dickson, P. A., Montgomery, G. W., Heath, A. C., Whitfield, J. B., et al. (2008). Association of the gastric alcohol dehydrogenase gene ADH7 with variation in alcohol metabolism. *Human Molecular Genetics, 17*, p. 179-189.

Crabb, D. W., Matsumoto, M., Chang, D., & You, M. (2004). Overview of the role of alcohol dehydrogenase and aldehyde dehydrogenase and their variants in the genesis of alcohol-related pathology. *Proceedings of the Nutrition Society, 63*(1), p. 49-63.

Edenberg, H. J. (2007). The genetics of alcohol metabolism - Role of alcohol dehydrogenase and aldehyde dehydrogenase variants. *Alcohol Research & Health, 30*(1), p. 5-13.

Ehlers, C. L. (2007). Variations in ADH and ALDH in southwest California Indians. *Alcohol Research & Health, 30*(1), p. 14-17.

Farres, J., Wang, X. P., Takahashi, K., Cunningham, S. J., Wang, T. T., & Weiner, H. (1994). Effects of changing glutamate-487 to lysine in rat and human liver mitochondrial aldehyde dehydrogenase – a model to study human (oriental type) class-2 aldehyde dehydrogenase. *Journal of Biological Chemistry, 269*(19), p. 13854-13860.

Iwahashi, K., Ameno, K., Kinoshita, H., Nakamura, K., Miyatake, R., Suwaki, H., et al. (1996). CYP2E1, ALDH2 and ADH2 genotypes and blood ethanol elimination kinetics. *Clinica Chimica Acta, 255*(1), p. 85-87.

Mulligan, C. J., Robin, R. W., Osier, M. V., Sambuughin, N., Goldfarb, L. G., Kittles, R. A., et al. (2003). Allelic variation at alcohol metabolism genes (ADH1B, ADH1C, ALDH2) and alcohol dependence in an American Indian population. *Human Genetics, 113*(4), p. 325-336.

Novoradovsky, A., Tsai, S. J. L., Goldfarb, L., Peterson, R., Long, J. C., & Goldman, D.

(1995). Mitochondrial aldehyde dehydrogenase polymorphism in Asian and American – Indian populations – detection of new ALHD2 alleles. *Alcoholism-Clinical and Experimental Research, 19*(5), p. 1105-1110.

Segal, B., Hesselbrock, V. M., & Linn, R. (1998). Polymorphisms of ADH and ALDH genes among Alaska natives entering treatment for alcoholism. *Alcoholism Clinical and Experimental Research, 22*(3 ABSTR. SUPPL.), 116A.

Trujillo, K. A., Castaneda, E., Martinez, D., & Gonzalez, G. (2006). Biological research on drug abuse and addiction in Hispanics: Current status and future directions. *Drug and Alcohol Dependence, 84*, p. S17-S28.

Wall, T. L., Carr, L. G., & Ehlers, C. L. (2003). Protective association of genetic variation in alcohol dehydrogenase with alcohol dependence in native American mission Indians. *American Journal of Psychiatry, 160*(1), p. 41-46.

Wan, Y. J. Y., Poland, R. E., & Lin, K. M. (1998). Genetic polymorphism of CYP2E1, ADH2, and ALDH2 in Mexican-Americans. *Genetic Testing, 2*(1), p. 79-83.

Werner Goedde, H., & Agarwal, D. P. (1990). Pharmacogenetics of aldehyde dehydrogenase (ALDH). *Pharmacology & Therapeutics, 45*(3), p. 345-371.

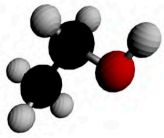
Yoshihara, E., Ameno, K., Nakamura, K., Ameno, M., Itoh, S., Ijiri, I., et al. (2000). The effects of the ALDH2*1/2, CYP2E1 C1/C2 and C/D genotypes on blood ethanol elimination. *Drug and Chemical Toxicology, 23*(2), p. 371-379.

Web-based References

Drosophila Genetics in the Classroom
http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1205791

Drunken flies mimic human behavior:
http://www.hhmi.org/genesweshare/b310.html

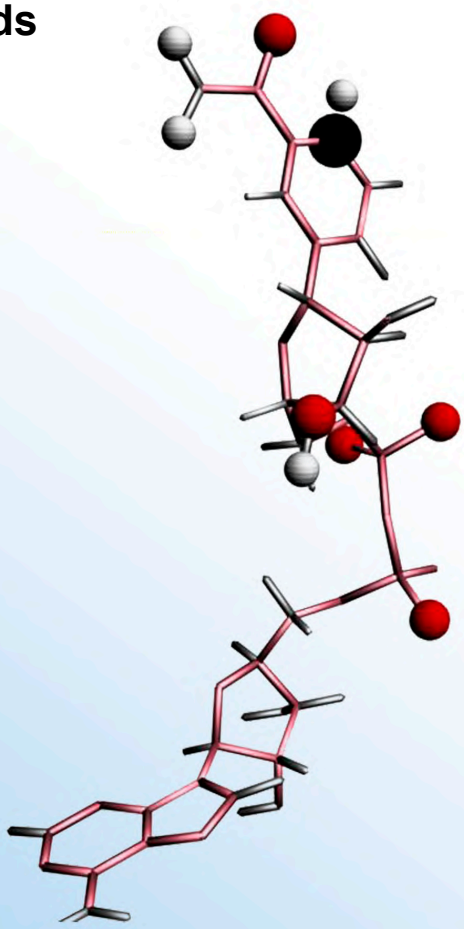
Appendix

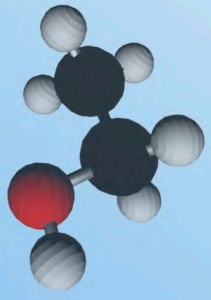


National Science Education Standards

Supplementary Content

Answers to Assessment Questions





National Science Education Standards

Chapter 1

Description of the chapter

Alcohol, which is consumed by some as a beverage, is actually an organic molecule that is known as ethanol in the scientific world. Students will learn about the structure of ethanol, ethanol's unique characteristics, and what happens to ethanol immediately after it is consumed. In the final part of the chapter, students will learn why ethanol absorption differs depending on whether a person is male or female. The biology and chemistry concepts in this chapter include:

1) structure of organic molecules (i.e., alcohols)

2) polar versus non-polar compounds

3) structure and properties of biological membranes

4) human digestive system (GI tract and the liver)

5) transport of a chemical (alcohol) across a biological membrane (passive diffusion and concentration gradient)

National Science Education Content Standards

- **CA1** - Abilities necessary to do scientific inquiry
- **CA23** - Formulate and revise scientific explanations and models using logic and evidence
- **CA25** - Communicate and defend a scientific argument
- **CB2** - Structure and properties of matter
- **CB5** - Conservation of energy and increase in disorder
- **CB20** - Atoms interact with one another by transferring or sharing electrons that are furthest from the nucleus. These outer electrons govern the chemical properties of the element

- **CB23** - The physical properties of compounds reflect the nature of the interactions among its molecules. These interactions are determined by the structure of the molecule, including the constituent atoms and the distances and angles between them.
- **CB25** - Carbon atoms can bond to one another in chains, rings, and branching networks to form a variety of structures, including synthetic polymers, oils, and the large molecules essential to life.
- **CC50** - All matter tends toward more disorganized states. Living systems require a continuous input of energy to maintain their chemical and physical organizations. With death, and the cessation of energy input, living systems rapidly disintegrate.
- **CG3** - Historical perspectives

Chapter 2

Description of the chapter

What happens once alcohol enters the bloodstream? Where does it go? This chapter describes the distribution pathway of ethanol – how it reaches the heart, how it travels through the heart, and how it eventually reaches the brain. The chapter also illustrates gender differences in ethanol concentration in the blood when the same amount of alcohol is consumed. The biology and chemistry concepts in this chapter include:

1) structural properties and the role of capillaries

2) human circulatory system

3) human digestive system (GI tract and the liver)

4) state of equilibrium

5) polar versus non-polar compounds

6) body mass and composition (fat and water percentages)

National Science Education Content Standards

- **CA1** - Abilities necessary to do scientific inquiry
- **CA22** - Use technology and mathematics to improve investigations and communications
- **CA23** - Formulate and revise scientific explanations and models using logic and evidence
- **CA25** - Communicate and defend a scientific argument
- **CB2** - Structure and properties of matter

- **CB5** - Conservation of energy and increase in disorder
- **CB20** - Atoms interact with one another by transferring or sharing electrons that are furthest from the nucleus. These outer electrons govern the chemical properties of the element.
- **CC1** - The cell
- **CC10** - Cells have particular structures that underlie their functions. A membrane that separates it from the outside world surrounds every cell. Inside the cell is a concentrated mixture of thousands of different molecules which form a variety of specialized structures that carry out such cell functions as energy production, transport of molecules, waste disposal, synthesis of new molecules, and the storage of genetic material.
- **CC11** - Most cell functions involve chemical reactions. Food molecules taken into cells react to provide the chemical constituents needed to synthesize other molecules. Both breakdown and synthesis are made possible by a large set of protein catalysts, called enzymes. The breakdown of some of the food molecules enables the cell to store energy in specific chemicals that are used to carry out the many functions of the cell.
- **CC50** - All matter tends toward more disorganized states. Living systems require a continuous input of energy to maintain their chemical and physical organizations. With death, and the cessation of energy input, living systems rapidly disintegrate.

Chapter 3

Description of the chapter

This chapter describes how our bodies use a process known as metabolism to get rid of the ethanol that we consume. To eliminate the ethanol, two oxidation reactions take place in the liver. The chapter provides students with the necessary background information on oxidation, and then discusses each of the reactions in great detail. Kinetic characteristics of enzymes are also covered. The biology and chemistry concepts in this chapter include:

1) basic concepts of oxidation and reduction

2) oxidation reactions (metabolism of alcohol)

3) enzymes as catalysts of biological reactions (e.g., alcohol dehydrogenase and aldehyde dehydrogenase)

4) cell organelles

5) kinetic characteristics of enzymes (capacity and affinity)

National Science Education Content Standards

- **CA1** - Abilities necessary to do scientific inquiry
- **CA23** - Formulate and revise scientific explanations and models using logic and evidence
- **CB2** - Structure and properties of matter
- **CB3** - Chemical reactions
- **CB5** - Conservation of energy and increase in disorder
- **CB20** - Atoms interact with one another by transferring or sharing electrons that are furthest from the nucleus. These outer electrons govern the chemical properties of the element.
- **CB22** - Bonds between atoms are created when electrons are paired up by being transferred or shared. A substance composed of a single kind of atom is called an element. The atoms may be bonded together into molecules or crystalline solids. A compound is formed when two or more kinds of atoms bind together chemically.
- **CB25** - Carbon atoms can bond to one another in chains, rings, and branching networks to form a variety of structures, including synthetic polymers, oils, and the large molecules essential to life.
- **CB32** - A large number of important reactions involves the transfer of either electrons (oxidation/reduction reactions) or hydrogen ions (acid/base reactions) between reacting ions, molecules, or atoms. In other reactions, chemical bonds are broken by heat or light to form very reactive radicals with electrons ready to form new bonds. Radical reactions control many processes such as the presence of ozone and greenhouse gases in the atmosphere, burning and processing of fossil fuels, the formation of polymers, and explosions.
- **CB34** - Catalysts, such as metal surfaces, accelerate chemical reactions. Protein molecules called enzymes catalyze chemical reactions in living systems.
- **CC1** - The cell
- **CC11** - Most cell functions involve chemical reactions. Food molecules taken into cells react to provide the chemical constituents needed to synthesize other molecules. Both breakdown and synthesis are made possible by a large set of protein catalysts, called enzymes. The breakdown of some of the food molecules enables the cell to store energy in specific chemicals that are used to carry out the many functions of the cell.

Chapter 4

Description of the chapter

Since each person has a unique genetic makeup, does this mean that the enzymes involved in ethanol metabolism can also be different for different people? The answer to this question is "yes." Students will learn about basic genetic concepts and about how different enzyme polymorphisms play a role in determining an individual's risk for alcoholism as well as a population's risk for alcoholism. The biology and chemistry concepts in this chapter include:

1) deoxyribonucleic acid (DNA) structure

2) transcription and translation

3) genetic polymorphism

4) protein expression and function

5) enzyme capacity and affinity

National Science Education Content Standards

- **CA1** - Abilities necessary to do scientific inquiry
- **CA23** - Formulate and revise scientific explanations and models using logic and evidence
- **CA22** - Use technology and mathematics to improve investigations and communications
- **CA25** - Communicate and defend a scientific argument
- **CA26** - Understand about scientific inquiry
- **CC1** - The cell
- **CC10** - Cells have particular structures that underlie their functions. A membrane that separates it from the outside world surrounds every cell. Inside the cell is a concentrated mixture of thousands of different molecules which form a variety of specialized structures that carry out such cell functions as energy production, transport of molecules, waste disposal, synthesis of new molecules, and the storage of genetic material.
- **CC11** - Most cell functions involve chemical reactions. Food molecules taken into cells react to provide the chemical constituents needed to synthesize other molecules. Both breakdown and synthesis are made possible by a large set of protein catalysts, called enzymes. The breakdown of some of the food molecules enables the cell to store energy in specific chemicals that are used to carry out the many functions of the cell.
- **CC12** - Cells store and use information to guide their functions. The genetic information stored in DNA is used to direct the synthesis of the thousands of proteins that each cell requires.
- **CC13** - Cell functions are regulated. Regulation occurs both through changes in the activity of the functions performed by proteins and through the selective expression of individual genes. This regulation allows cells to respond to their environment and to control and coordinate cell growth and division.
- **CG1** - Science as a human endeavor
- **CG2** - Nature of scientific knowledge
- **CG3** - Historical perspectives
- **CG12** - Scientists are influenced by societal, cultural, and personal beliefs and ways of viewing the world. Science is not separate from society but rather science is a part of society.
- **CC13** - Cell functions are regulated. Regulation occurs both through changes in the activity of the functions performed by proteins and through the selective expression of individual genes. This regulation allows cells to respond to their environment and to control and coordinate cell growth and division.

- **CG1** - Science as a human endeavor
- **CG2** - Nature of scientific knowledge
- **CG3** - Historical perspectives
- **CG12** - Scientists are influenced by societal, cultural, and personal beliefs and ways of viewing the world. Science is not separate from society but rather science is a part of society.
- **CG20** - Science distinguishes itself from other ways of knowing and from other bodies of knowledge through the use of empirical standards, logical arguments, and skepticism, as scientists strive for the best possible explanations about the natural world.
- **CG21** - Scientific explanations must meet certain criteria. First and foremost, they must be consistent with experimental and observational evidence about nature, and must make accurate predictions, when appropriate, about systems being studied. They should also be logical, respect the rules of evidence, be open to criticism, report methods and procedures, and make knowledge public. Explanations on how the natural world changes based on myths, personal beliefs, religious values, mystical inspiration, superstition, or authority may be personally useful and socially relevant, but they are not scientific.
- **CG22** - Because all scientific ideas depend on experimental and observational confirmation, all scientific knowledge is, in principle, subject to change as new evidence becomes available. The core ideas of science such as the conservation of energy or the laws of motion have been subjected to a wide variety of confirmations and are therefore unlikely to change in the areas in which they have been tested. In areas where data or understanding are incomplete, such as the details of human evolution or questions surrounding global warming, new data may well lead to changes in current ideas or resolve current conflicts. In situations where information is still fragmentary, it is normal for scientific ideas to be incomplete, but this is also where the opportunity for making advances may be greatest.

Supplementary Content

A Primer on Redox Reactions

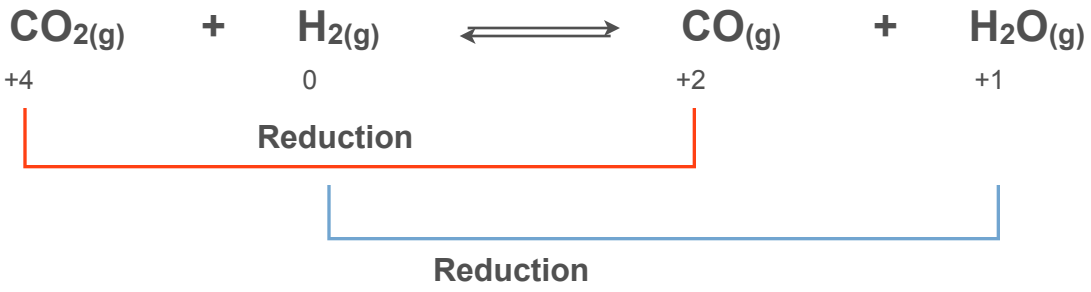
A redox reaction takes into account both the oxidation and reduction that occur simultaneously during the transfer of electrons from one atom (or molecule) to another.

The two parts to a redox reaction can be summarized in the following table:

Oxidation	**Reduction**
Reductant $\longrightarrow$ product + e^-	Oxidant + e^- $\longrightarrow$ product
Loss of electrons	Gain of electrons
Oxidation number increases	Oxidation number decreases

A handy mnemonic for redox reactions is **OILRIG**: **O**xidation **I**s **L**oss (of electrons), **R**eduction **I**s **G**ain (of electrons).

To gain a better understanding of redox reactions, let us examine the following reaction:



In this reaction, carbon dioxide (CO_2) is reduced to carbon monoxide (CO) because it loses an oxygen; its oxidation state decreases from +4 to +2. On the contrary, the hydrogen molecule (H_2) is oxidized to form water (H_2O) because it gains an oxygen; its oxidation state increases from 0 to +1. CO_2 in this reaction is the oxidizing agent, or **oxidant**, because it causes oxidation and becomes reduced in the process. H_2 is the reducing agent, or **reductant**, because it causes reduction and becomes oxidized.

For advanced students

Calculating the oxidation state

Calculating the oxidation state, or number, is not as hard as it may seem. Consider that the oxidation state of a free element is zero, and the oxidation state of an atom or simple ion is the same as the ionic charge on the atom or ion. For example, the oxidation state of Cl is 0, but the oxidation state of the Cl as an ion (Cl^-) is -1.

In a neutral molecule the sum of the oxidation states of all the atoms must equal zero. However, in an ionic molecule, the sum of the oxidation states of all atoms must add up to the charge on the ionic molecule. Calculating the oxidation state of an atom in a molecule can be done with or without a Lewis structure. Using the Lewis structure method, the oxidation state of an atom is calculated by subtracting the number of valence electrons shown in the Lewis Structure from the number of valence electrons the atom would have in its neutral state. As one can imagine, the process of drawing out Lewis structures can become tedious, so when a Lewis structure is not available we use some typical rules to help us in our calculations. In addition to the rules mentioned above, the following rules are also relevant:

1. The oxidation state of oxygen is -2, except in peroxides where it is -1
2. The oxidation state of hydrogen is +1, except when bonded to binary metals (lithium, sodium, calcium, etc.), giving an oxidation state of -1
3. The oxidation state of fluorine is -1
4. The oxidation state for alkali metals is +1
5. The oxidation state for alkaline earth metals is +2
6. The oxidation state of halogens is -1, except when bonded to oxygen or a more electronegative atom

Stoichiometry in Redox Reactions

Stoichiometry is the area of chemistry that focuses on the quantitative relationship among the substances in a chemical reaction. Stoichiometry is based on the law of conservation of mass, the law of constant composition, and the law of multiple proportions. It is often used in the following circumstances:

- To balance equations
  In chemical reactions, such as redox reactions, the number of moles of each element must be equivalent on the right and left side of the equation.

- To describe molar proportions
  In a compound, it is useful to know the number of moles of one element relative to another. For example, in H_2O the molar proportion of Hydrogen to Oxygen is 2:1.

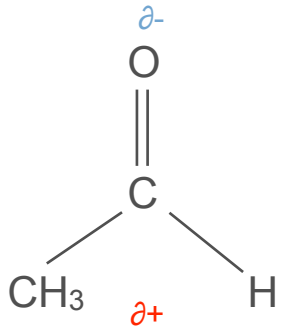
- To calculate conversions
  In chemistry, we often need to convert from one unit of measure to another. For example, if we wanted to determine how many grams are in X number of moles, then we would use stoichiometric ratios. Each of the above circumstances is used to qualitatively and quantitatively describe redox reactions.

A detailed look at acetaldehyde oxidation

Unlike the oxidation of ethanol, acetaldehyde oxidation involves water.
Water exists naturally in equilibrium among its neutral (water, H_2O), acidic (hydronium, H_3O^+), and basic (hydroxide, OH^-) states:

$$H_3O^+ \rightleftharpoons H_2O \rightleftharpoons OH^-$$

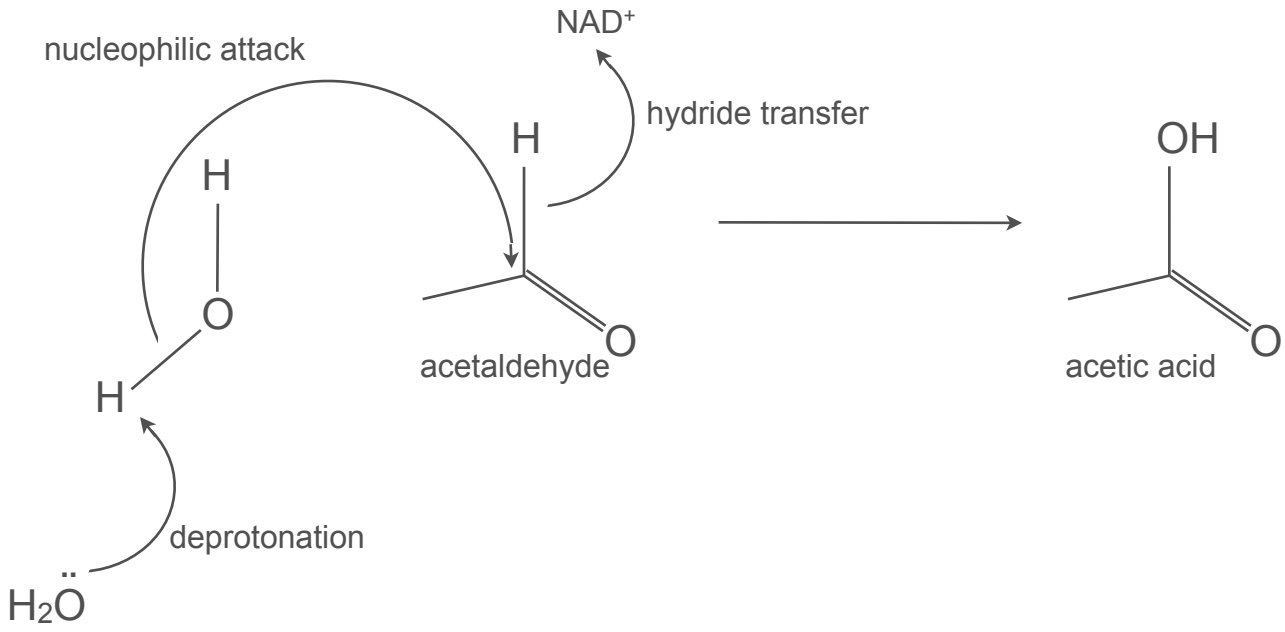
Because opposite charges attract, hydronium is attracted to negatively charged atoms, and hydroxide is attracted to positively charged atoms. In acetaldehyde, the oxygen that is double bonded to the carbon (a carbonyl bond) is slightly negative, while the carbonyl carbon is slightly positive. The partial charge on the carbon and oxygen occurs because oxygen is significantly more electronegative than carbon, and the double bond between these two atoms exacerbates the effect of this difference in electronegativity.



Thus, hydronium is somewhat attracted to the carbonyl oxygen, while hydroxide is somewhat attracted to the carbonyl carbon. While the exact mechanism of ALDH-assisted aldehyde oxidation is still uncertain, there are two probable mechanisms. One is more favored in basic conditions, in which there is more hydroxide in solution than hydronium; the other is more favored in acidic conditions, in which there is more hydronium.

Studies indicate that basic conditions are more likely to exist during oxidation of acetaldehyde. Hydroxide ions present in the mitochondria can "attack" the positively charged carbonyl carbon (the carbon double-bonded to oxygen). In other words, the negative oxygen in hydroxide seeks to form a bond with the partially positive carbon. Because the carbon cannot violate the octet rule, it temporarily "relinquishes" its double bond with the adjacent oxygen. For a short time, the carbon-oxygen connection of acetaldehyde becomes a single bond, and the oxygen becomes negatively charged.

As before, this state is highly unstable, and the lone pair of electrons on the carbonyl oxygen very quickly re-form the double bond with the carbonyl carbon. The carbonyl carbon then releases a hydride to NAD^+ that is present in the ALDH active site. The net result is the replacement of a hydrogen with a hydroxyl group. The newly formed acetic acid, along with NADH, then becomes free to leave the ALDH active site. The process repeats when another pair of NAD^+ and acetaldehyde molecules enter the ALDH active site. The oxidation reaction for acetaldehyde is written typically below:



As with the oxidation of ethanol, the oxidation of acetaldehyde can be written as two half-reactions or one complete reaction.

Half reactions:

water + acetaldehyde $\longrightarrow$ acetic acid + H^+ + H^-

H^- + NAD^+ $\longrightarrow$ NADH

complete reactions:

water + acetaldehyde + NAD^+ $\longrightarrow$ acetic acid + NADH + H^+

Answers to Assessment Questions

Multiple Choice Questions

Chapter 1

1. B 2. D 3. D

Chapter 2

1. B 2. D 3. C 4. A

Chapter 3

1. E 2. C 3. B 4. B

Chapter 4

1. A 2. B 3. C

Open-Ended Questions

1. Ethanol enters the bloodstream from the capillaries that line the GI tract. Similar to the way that ethanol crosses biological membranes, ethanol is easily able to cross the thin capillary walls primarily due to its small size. The capillaries that line the GI tract converge into the portal vein, which then branches out into another system of capillaries inside the liver. Once in the liver, some of the ethanol is metabolized in the hepatocytes and the rest is carried by the venous system to the right side of the heart. The right side of the heart then directs the venous blood to the lungs where a tiny amount of the ethanol is eliminated as a gas during exhalation. The ethanol that remains in the blood returns to the left side of the heart, which sends the blood to the entire body via the arteries. Much of the ethanol dissolved in the blood ends up entering the brain where it interferes with neuronal function and thus causes people to become “drunk”.

2. NAD+ is a crucial coenzyme in biological oxidation reactions. In the first step of metabolism, it actually performs the oxidation of ethanol to acetaldehyde. It does this by binding to the active site of the ADH enzyme. During the oxidation of ethanol, the hydrogen atom of the hydroxyl (-OH) group forms a hydrogen bond with an amino acid on the active site; however, almost immediately, a proton transfer occurs and the amino acid breaks the O-H bond by taking the H+ from the oxygen. The lone oxygen now prefers to form a double bond with the carbon in the hydrocarbon chain of ethanol. However, to remain stable, the carbon on the ethanol must then lose one of its extra bonds. Thus, ethanol loses a hydride (hydrogen atom with a pair of electrons) and becomes acetaldehyde. The hydride is accepted by NAD+; therefore, NAD+ is reduced to NADH and its oxidation state is decreased.

FAD+ is also a coenzyme that helps in biochemical reactions and allows the oxidation reaction to occur. By acting as an oxidizing agent, FAD+ accepts a hydride and is reduced to FADH.

3. A hydrogen bond is a bond formed between a hydrogen atom and an electronegative atom such as nitrogen, oxygen, or fluorine. In the first step of metabolism during the proton transfer, a hydrogen bond is formed when the ethanol terminal H (from the OH bond) forms another bond with the electronegative O atom within an amino acid in the ADH active site. As the electronegative O on the ADH pulls on the proton, the new hydrogen bond becomes broken; the H originally associated with the ethanol OH now becomes bound covalently to the O on the ADH amino acid. In a covalent bond, two atoms share their valence electrons so that both atoms have complete outer shells. Another example of covalent bond formation in the metabolism of ethanol is after the loss of the proton. A covalent bond forms when the lone pairs of electrons on the carbonyl oxygen form a double bond with the carbonyl carbon.
Covalent bonds are stronger than hydrogen bonds because hydrogen can help stabilize the reaction by easily releasing itself from its weak bond.

Students can also cite the following examples:

- During the formation of the hydride, the double, covalent C-O bond is temporarily broken when the negative O in the hydroxide tries to form a bond with the carbonyl carbon
- A covalent bond forms when a carbon atom on the NAD+ accepts a hydrogen to become NADH. Eventually this covalent bond is broken when NADH is oxidized back to NAD+.

4. Students should recall from Chapters 2 and 3 that metabolism of ethanol occurs in the hepatocytes; however not all of the ethanol can be metabolized because there are only a finite number of enzyme molecules available. It is also important for students to remember that the ethanol that is *not* metabolized continues to move through the venous system, return to the heart, and up to the brain, causing intoxication. Thus, if Mark's genetic mutation causes ADH to catalyze his metabolic process much slower than most males, then he will have a larger proportion of ingested alcohol that circulates in his blood, so his blood alcohol level will be higher compared to other males with faster metabolism. In addition, Mark will experience a greater degree of intoxication, earlier.