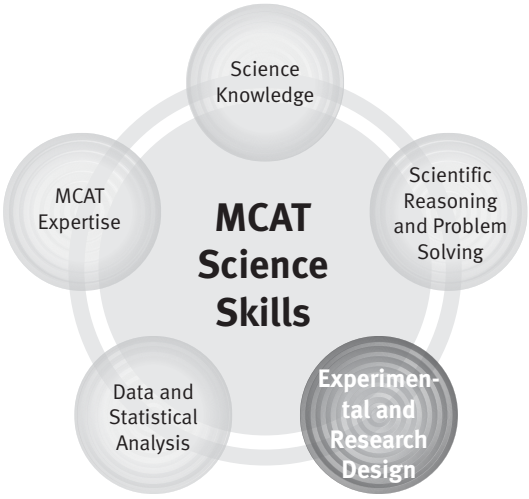
Advanced Experimental Design

In this lesson, you'll learn to:

- Make and identify valid conclusions that can be drawn from research results
- Relate the results of a study to real-world situations



Science Topics

- The Excretory System
- The Immune System
- The Lymphatic System
- The Circulatory System
- The Digestive System
- DNA as Genetic Material/Genetic Analysis
- Mendelian Concepts

LESSON 3.4, LEARNING GOAL 1:

- Identify and make valid conclusions that can be drawn from research results

Questioning the Validity of Conclusions

Study 1
Cholecystitis, or inflammation of the gall bladder, occurs when there is an obstruction of the cystic duct. One treatment is surgical removal of the gallbladder, or cholecystectomy. There are two options for surgical intervention: 1) an open exploratory surgery or 2) a closed laparoscopic surgery facilitated by the use of a camera. A group of gastroenterologists conduct a series of randomized controlled trials to examine the effectiveness of the two interventions in the general population. A total of 478 patients were asked to participate in the study. A total of 352 were randomized into one of the two intervention groups. Those that were not randomized were either too frail to undergo an open surgical procedure or displayed advanced gall bladder disease, both of which required laparoscopic intervention. After gathering postoperative outcome data, the doctors found that laparoscopic surgery—the intervention utilizing the camera—is the preferred treatment method for cholecystitis in the general population.

Looking at the table below, what concerns you about their conclusion?

	Men (n)	Women (n)	Age (average)
Randomized Group	41	281	43
Non-Randomized Group	60	66	58

Table 1. Demographics of cholecystectomy study.

Study 2
Huntington's disease is a neurodegenerative disease that is transmitted through autosomal dominant inheritance. Patients do not exhibit symptoms until age 35–44 years. Given these facts and the chance that 50 to 100 percent of the affected patient's offspring will have the trait, genetic screening is recommended for the children of affected patients. A neurologist tests the effectiveness of a digital intervention to encourage genetic testing by gathering the email addresses of affected patients who visit as well as those of their adult children. Patients and their children were randomized into either a control group, who received a letter about genetic screening, and an intervention group, who received an email from their physician about genetic testing. Of the 34 patients in the intervention group, eight viewed the email and two set up an appointment to discuss genetic testing. Only one of the seven adult children of affected patients viewed the email and none signed up for genetic testing. Given these results, the neurologist concluded that email intervention is not an effective method for raising awareness about genetic testing.

What aspect of the findings might lead us to question the neurologist's conclusion?

This lesson continues on the next page ▶ ▶ ▶

Making Valid Conclusions—Practice Passage (Questions 1–5)

In hemophilia, the blood fails to coagulate and its two types, A and B, result from the absence of clotting factors VIII and IX, respectively. Inheritance follows an X-linked recessive trait. In the past, patients were treated with clotting factors purified from the blood of several donors. In the 1990s, however, scientists were able to make recombinant factors to replace patients' missing factors.

A researcher replicates the findings of the original experiment, which produced recombinant factor VIII. She uses the plasmid pUC18 as a vector for FVIII because the plasmid contains *bla*, a gene that codes for beta-lactamase and confers resistance to the antibiotic ampicillin.

Experiment 1
The researcher treats the plasmid pUC18 with the restriction enzyme *Scal* and then proceeds to introduce the plasmid to *E. coli*. She incubates the bacteria at 35°C in nutrient complex media and applies ampicillin after 48 hours.

Experiment 2
Deciding to run the experiment again, the researcher uses the restriction enzyme *Lgul* to treat the plasmid pUC18 (see Table 1).

	Control Plates	pUC18 plates
Scal	---	---
Lgul	---	+++

Table 1. Results for Experiments 1 and 2.

Some patients receiving recombinant clotting factor treatment form antibodies, thereby inhibiting the function of the exogenous factor. While it is unclear why this only occurs in some cases, these patients generally exhibit tolerance with repeated administration of the recombinant factor and may eradicate the inhibitors over time.

Experiment 3
The researcher is curious to find out why only a small percentage of hemophiliacs develop recombinant factor inhibitors. After conducting a series of sequencing studies, she obtains the following results:

Mutation Type	Missense	Nonsense	Frameshift +	Frameshift –
Inhibitor Prevalence %	5	30	15	41
Tolerance Rate	0.9	0.45	0.82	0.3

Table 2. Prevalence of mutation types in hemophiliacs with inhibition.

Passage Outline

P1.

P2.

Expt 1.

Expt 2.

Table 1.

P3.

Expt 3/Table 2.

1. Based on the results of the experiments conducted by the researcher, which of the following best explains the outcome of Experiment 1?

A. The *E. coli* were not able to grow because they required nutrient complex media.
B. The pUC18 plasmid was not able to replicate at such high temperatures.
C. *Scal* spliced *bla*.
D. *Lgul* spliced FVIII.

2. Which of the following is the most likely reason recombinant clotting factor VIII would be considered preferable to previous methods of obtaining factor VIII?

A. The strain of *E. coli* used in newer methods is less toxic to humans.
B. Purified factor VIII is more likely to transmit pathogens.
C. Recombinant factor VIII is less pure than previous methods.
D. Patients infected with *E. coli* can be given the antibiotic ampicillin.

3. The most probable reason some patients develop inhibitors to recombinant FVIII is:

A. the loss of a sequence coding for regulatory protein that limits the response of the immune system.
B. the addition of a sequence coding for the light chain of a specific antibody.
C. the substitution of one amino acid for another in an MHC protein.
D. a rearrangement in the long arm of an autosomal chromosome.

4. A student decides to repeat Experiments 1 and 2, but uses a Ti (tumor-inducing) plasmid, which does not have ampicillin resistance. How will this affect her research results?

A. The results will mimic those of the first researcher.
B. FVIII will fail to transform into the vector.
C. Her plates will be susceptible to bacterial infection.
D. She won't be able to determine if FVIII failed to be transformed.

5. Which of the following pieces of evidence would most solidly confirm the known inheritance pattern?

A. Affected individuals in every generation
B. The same number of sons and daughters affected in every family
C. The disease in the sons of two unaffected parents
D. There is no way to confirm the known inheritance pattern

KAPLAN TIP

Always remember to look at study design with a critical eye on Test Day, because incorrect implementation of a study will negate the validity of its findings.

LESSON 3.4, LEARNING GOAL 2:

- Relate the results of a study to real-world situations

Real-World Implications—Practice Passage (Questions 6–8)

Diabetes mellitus is a common disorder caused by either a lack of or an insensitivity to insulin, and results in patients having excess glucose in their blood. This excess glucose causes unwanted glycosylation reactions in some of the smallest and most vulnerable blood vessels in the body, including those of the eyes, feet, and kidney. A common measure of kidney function, and a marker of the extent to which the vasa recta have been glycosylated, is creatinine levels in the blood (normal values 0.6–1.2 mg/dl), since damaged renal vessels will fail to filter waste products such as creatinine.

An endocrinologist wants to learn more about one of the renal complications of diabetes, diabetic nephropathy, in her practice and conducts the following two studies:

Study 1
First, the doctor surveys patient data from her two separate office locations and charts the data comparing creatinine levels and age.

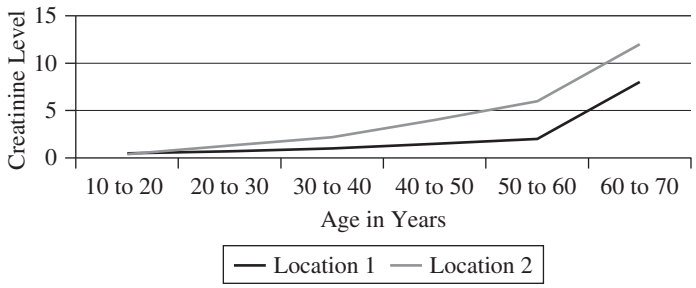


Figure 1. Creatinine levels *vs.* age for the two locations.

Study 2
Second, the doctor studies the effect of administering an ACE (angiotensin-converting enzyme) inhibitor, captopril, on creatinine levels in patients with documented prerenal disease due to diabetes. The results are shown below:

	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Diabetic NO Captopril	3 mg/dl	3.5 mg/dl	4.2 mg/dl	4.8 mg/dl	5.0 mg/dl	5.3 mg/dl
Diabetic WITH Captopril	2.8 mg/dl	3.0 mg/dl	3.1 mg/dl	3.1 mg/dl	3.0 mg/dl	3.1 mg/dl

Table 1. Concentration of creatinine in patients with and without captopril treatment.

6. Based on the results of Study 1, which of the following interventions makes the most sense for the doctor to implement?

A. A new policy lessening the number of required checkups at location 1.
B. Diabetic nephropathy counseling at location 1 for patients who are 20–40 years old.
C. Decreasing the glucose-controlling medications given to patients at location 2.
D. Diabetic nephropathy counseling at location 2 for patients who are 30–50 years old.

7. How can the doctor use the results of Study 2 to help the patients included in Study 1?

A. Treat only patients at location 2 with captopril.
B. Treat patients with a creatinine level of 2 mg/dl or higher with captopril.
C. Treat patients with a creatinine level of 1 mg/dl or lower with captopril.
D. The doctor cannot use the results of Study 2 to help the patients in Study 1.

8. Patients with diabetes are advised to limit their salt intake as one way to decrease the progression of diabetic nephropathy. This recommendation mirrors the findings in which one of the studies run by the endocrinologist? Why?

A. Study 1, because as time goes on patients lose the ability to process salt.
B. Study 2, because the patients not receiving captopril have lower blood sugar.
C. Study 2, because the patients receiving captopril are also lowering their blood pressure.
D. Study 1, because the patients at location 1 have lower blood pressure.

KAPLAN TIP

Relevancy is an important reason to conduct research at all, and the more relevant the research, the more applicable it will be to real-world situations.

LESSON 3.4 REVIEW

Making Valid Conclusions

Internal Validity

- Measures the degree to which a study answers the question it set out to answer.
- Factors affecting internal validity include anything that causes flaws in the design or data collection process (i.e., subject variability, attrition, sample size, and instrument sensitivity).

External Validity

- Measures the extent to which the study findings can be generalized.
- Factors affecting external validity include population characteristics, subject selection, the effect of time, and the effect of the research environment.

Once Validated

- One can feel comfortable about the conclusions reported.
- The findings can be applied to real-world situations in order to improve results in a given area.

Biology and Biochemistry 3: Advanced Experimental Design

PASSAGE I (QUESTIONS 1–6)

Hypercholesterolemia is the term used to describe abnormally high levels of cholesterol in the blood. Cholesterol is linked to proteins ("lipoproteins") in the blood; hypercholesterolemia describes high levels of lipoproteins, including high density lipoprotein (HDL) and very-low density lipoprotein (VLDL). However, it is high levels of low density lipoprotein (LDL) in the blood that is predominantly linked to an increased risk of atherosclerosis and heart disease.

While hypercholesterolemia can be caused by environmental factors, familial hypercholesterolemia (FH) is caused by mutant alleles of genes associated with LDL uptake from the blood. The most common cause of FH is dominant mutations in the LDL receptor (LDLR) gene. Normal LDL receptors are located in liver cells, where they bind LDL particles from the blood. After binding, both the receptor and the LDL are taken into the cell; the LDL particle is released into the cell to be metabolized, and the receptor returns to the cell surface to bind more LDL. Several different LDLR gene mutations exist that can cause problems with either the synthesis or proper function of the LDLR protein.

Another common cause of autosomal dominant FH is the ApoB gene. ApoB is the main protein component of LDL particles that forms the connection with LDLR. Mutant ApoB proteins are unable to bind to LDLR; therefore, the LDL particles remain in circulation. Finally, the LDLR adaptor protein ADH interacts with the LDL receptor within the cell, helping it to be taken into the cell after it has attached to an LDL particle. A diagram of the interaction between LDLR, ApoB, and ADH is shown in Figure 1. Autosomal recessive mutations in ADH inhibit the uptake of LDLR into the cell, thereby keeping the receptor from removing the particles from circulation. Typical plasma LDL levels from these mutations are shown in Table 1.

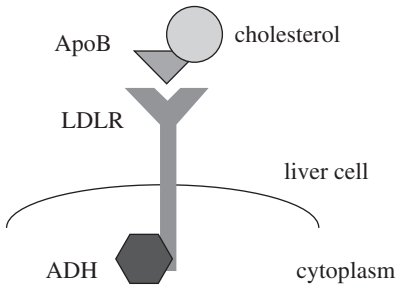


Figure 1. Interaction of ApoB, LDLR, and ADH.

Genotype	Blood LDL (mg/dL)
Wildtype	80
Heterozygous LDLR	170
Homozygous LDLR	450
Heterozygous ApoB	95
Homozygous ApoB	250
Heterozygous ADH	90
Homozygous ADH	200

Table 1. Average plasma LDL profiles of individuals who are either homozygous or heterozygous for alleles associated with familial hypercholesterolemia.

1. A child with FH exhibiting plasma LDL levels of 500 mg/dL is given a liver transplant from a wildtype individual and exhibits no improvement in plasma LDL after recovery. What is the most likely explanation for this occurrence?

A. The child is heterozygous for an ADH mutation.
B. Gene crossover occurs between the child's natural cells and the new liver cells, causing the liver to produce mutant LDLR protein.
C. The child is homozygous for an ApoB mutation with high expressivity.
D. The child eats fast food twice a week.

2. Individuals who are heterozygous for an LDLR gene mutation produce half the number of normal LDL receptors as a wildtype individual, while the other half are made by the mutant allele. This is an example of:

A. co-dominance.
B. penetrance.
C. incomplete dominance.
D. translocation.

3. There is a mutation in the main protein component of LDL particles that forms the connection with LDLR in a patient. How many copies of this mutation must this patient have?

A. One copy
B. Two copies
C. One or two copies
D. One, two, or three copies

4. A patient with parents who are both affected by familial hypercholesterolemia has fairly normal serum levels of FH. What are possible mutations that this patient could have?

I. Heterozygous ADH
II. Heterozygous ApoB
III. None

A. I only
B. II only
C. III only
D. I and II

5. A woman's parents, two brothers, and one other sister all suffer from FH. She marries a man who suffers from LH and one of their three children has FH. What is the most likely cause of the high cholesterol phenotype in this family?

A. LDLR mutation
B. ADH mutation
C. ApoB mutation
D. A or B

6. A daughter whose parents and one sibling do *not* suffer from FH has another sibling who does suffer from FH. What is probability that this daughter is a carrier?

A. 100%
B. 75%
C. 12.5%
D. 67%

PASSAGE II (QUESTIONS 1–6)

The intestines are known to have special structural features that allow them to function efficiently. These special features allow the intestines to maximize time for digestion and absorption.

Single-unit smooth muscle cells in the intestines are known to have gap junctions. These gaps allow the muscles to work in syncytia. Gap junctions also play an important part in tissue homeostasis because they allow for the exchange of ions, signaling molecules, nucleotides, and other small molecules between adjacent cells. Other organs, such as the stomach, are also known to have these features, but the esophagus and gall bladder do not.

Ions are reabsorbed through channels in the large intestine. For instance, Na^+ enters into the colon via an epithelial sodium channel. Similarly, to adjust the fluidity of the colon and to allow mixing and movement, Cl^- is secreted into the lumen. Na^+ and water follow the Cl^- into the lumen via the paracellular pathway.

Cancers of the small intestine are fairly rare. Factors that can protect against these types of cancers include having a lower bacterial and a higher alkaline pH in the small intestine. One other protective factor is the presence of benzpyrene hydroxylase, an enzyme that is thought to break down polycyclic aromatic hydrocarbons.

1. Smooth muscle gap junctions are not found in all organs, for instance in the esophagus or gall bladder. What could be a possible explanation for this?

A. The esophagus and gall bladder are not involved in secretion of substances.
B. The esophagus and gall bladder do not absorb anything.
C. The esophagus and gall bladder are not involved in digestion.
D. The esophagus and gall bladder have other similar features.

2. Which of the following would be the direct result of low sodium diet?

A. Increased reabsorption of water
B. Increased secretion of sodium
C. Increased expression of epithelial sodium channels
D. Decreased expression of epithelial sodium channels

3. Cholera is an infection caused by the bacterium *Vibrio cholerae*. It is known to increase cAMP to activate Cl^- secretory channels. Based on the passage, what would be the consequence of this pathology?

A. Increased fluid retention in the body
B. Increased sodium reabsorption
C. Increased potassium reabsorption
D. Loss of fluids from the body

4. Which of the following is the most likely molecule to pass through a gap junction that connects two cells of the intestine?

A. An antibody
B. A hormone
C. An amino acid
D. An enzyme

5. Why would having the enzyme benzpyrene hydroxylase present in the small intestine potentially protect against cancer?

A. Benzpyrene hydroxylase decreases the amount of polycyclic aromatic hydrocarbons in the lumen, which causes cancer.
B. Benzpyrene hydroxylase increases the amount of polycyclic aromatic hydrocarbons in the lumen, which causes cancer.
C. Benzpyrene hydroxylase increases the amount of Cl^- in the lumen, which causes cancer.
D. Benzpyrene hydroxylase decreases the amount of Cl^- in the lumen, which causes cancer.

6. A mutation causes the paracellular pathway in the small intestine to function in a decreased capacity. What effect would this have on the consistency of the material within the lumen of the colon?

A. Increased Na^+ in the lumen
B. Increased water in the lumen
C. Increased fluidity of the material in the lumen
D. Decreased fluidity of the material in the lumen

PASSAGE III (QUESTIONS 1–5)

The major histocompatibility complex (MHC) is a protein complex found on the surface of most cells in the body. When proteins are degraded within a cell, small pieces of these proteins, called epitopes, are displayed on the cell surface via the MHC complex. Non-host cells are digested by phagocytic cells of the immune system; phagocytic immune cells that then present foreign antigens at their MHC complex include macrophages and dendritic cells. MHCs are critical in the development of adaptive immunity, as they are responsible for presenting foreign proteins to lymphocytes, which then retain knowledge of the epitopes.

MHC II is expressed exclusively on the surface of antigen-presenting cells, including macrophages, B-cells, and dendritic cells. When these phagocytic cells take up and process an antigen, they display resulting epitopes on the surface at their MHC II complexes. Epitopes at these complexes are recognized by naïve helper T-cells which, during maturation in the thymus, are programmed to only recognize non-self epitopes. When they are exposed to these epitopes, the helper T-cell can then differentiate into a memory T-cell (which retains knowledge of the epitope) or an effector T-cell (which stimulates other immune cells to function, including cytotoxic T-cells).

MHC I molecules are expressed on the surface of nucleated cells in humans. These complexes present antigens on the cell surface, which are recognized by cytotoxic T-cells. If the antigen is non-self and is recognized by the T-cell receptor at the surface of the cytotoxic T-cell, the T-cell will cause the infected host cell to die.

Most of the multiple proteins subunits that make up MHC complexes are encoded by genes located on chromosome 6. Diagrams of the MHC complexes are shown in Figure 1.

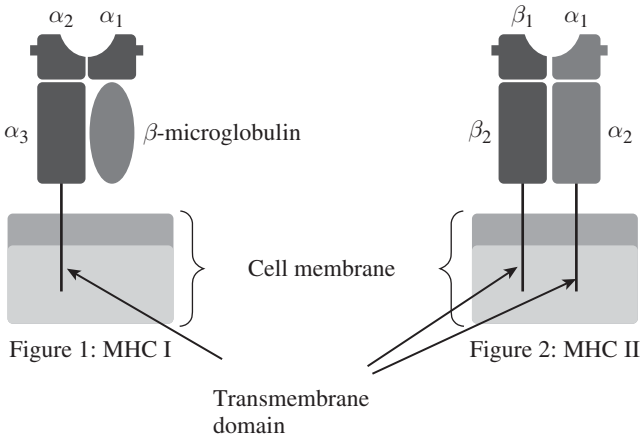


Figure 1. MHC complexes.

1. Which of the following cell types is unable to initiate an immune response through the use of an MHC complex?

A. Adipocytes
B. Erythrocytes
C. Spermatocytes
D. Myocytes

2. The products of both alleles of the genes for the alpha and beta subunits of the MHC complexes are expressed equally within the complex of an individual. This is an example of:

A. incomplete dominance.
B. genetic drift.
C. co-dominance.
D. evolution.

3. What is the most plentiful lymphocyte present at the site of a bacterial infection immediately following the introduction of these organisms to the system?

A. Helper T-cells
B. Natural killer cells
C. B-cells
D. Macrophages

4. An individual has mutations in both alleles for the alpha 3 subunit, which makes then non-functional. This individual is most likely to be:

A. unable to produce a humoral immune response.
B. unable to present MHC I at the cell surface.
C. unable to produce lymphocytes.
D. unable to induce the production of memory T-cells.

5. Which of the following would most likely result in a decreased immune response?

A. A mutation that causes increased expression of MHC II complexes on dendritic cells.
B. A mutation rendering the transmembrane domain of the MCH II complex nonfunctional.
C. A mutation that includes the MHC I complexes on non-nucleated cell surfaces.
D. A drug that increases the proliferation of macrophages within the body.

PASSAGE IV (QUESTIONS 1–5)

Excessive accumulation of interstitial (extravascular) fluid in tissues is known as edema. Although edema can be caused by a wide variety of disorders, its character and location varies with the particular illness, which makes it a valuable diagnostic indicator. Abnormal capillary dynamics may result in edema via one of four general mechanisms, which are described below.

Mechanism I
The most frequent cause of edema is high capillary blood pressure, which results in excessive movement of fluid into tissue spaces. Continuous overexpansion of extracellular tissue space gradually compromises its elastic network and eventually forms large fluid reservoirs. Elevated capillary blood pressure can also cause fluid to leak into various natural body cavities, such as the peritoneal cavity.

Mechanism II
Another common cause of edema is a decrease in plasma protein concentration, especially that of albumin. Plasma proteins are produced by the liver and then released into the blood. A decrease in plasma protein concentration causes a decrease in plasma osmotic pressure, which leads to a loss of fluid retention in the capillaries.

Mechanism III
The most severe type of edema results from lymphatic obstruction, which can seriously impede the drainage of proteins from extracellular spaces. The two common causes of lymphatic obstruction are surgical removal of regional lymph nodes (which routinely accompanies excision of a malignant tumor) and infection of the lymph nodes with the larvae of certain tropical parasites (which produces inflammatory lesions and eventually results in permanent scarring).

Mechanism IV
Edema can also arise from abnormally high capillary porosity, which leads to leakage of proteins and excess fluid out of the capillary lumen. For instance, certain vasoactive substances, such as histamine, can make capillaries leaky by acting directly on specific endothelial receptors.

1. In addition to causing edema, which of the four mechanisms would most likely decrease the body's resistance to local infection?

A. Mechanism I
B. Mechanism II
C. Mechanism III
D. Mechanism IV

2. Based on information in the passage, which of the following conditions would NOT be expected to cause edema?

A. Decreased fluid reabsorption by kidneys
B. Decreased lymphatic fluid flow
C. Increased protein excretion in urine
D. Increased permeability of capillary endothelium

3. Based on the fact that Mechanism II and Mechanism IV both cause edema by way of decreased plasma protein concentration, it could be concluded that:

A. capillaries are always fully permeable to albumin.
B. interstitial fluid albumin concentration is normally greater than plasma albumin concentration.
C. interstitial fluid albumin concentration is normally less than plasma albumin concentration.
D. histamine decreases blood vessel permeability.

4. Cortisol, a steroid hormone, has been shown to enhance the activity of liver enzymes required for protein synthesis. Based on this information, would cortisol administration be an effective treatment for a patient suffering from edema?

A. Yes, because cortisol would increase the concentration of plasma proteins and thus enhance fluid retention in the capillaries.
B. Yes, because cortisol would decrease capillary blood pressure.
C. No, because cortisol would decrease the concentration of plasma proteins by increasing metabolic rate.
D. No, because cortisol would increase the risk of lymph node infection.

5. Which of the four characteristics of inflammation is histamine likely responsible for, based on information in the passage?

A. Pain
B. Redness
C. Swelling
D. Heat