Eating for Health: A Look at How Diet can Affect the Immune System

Amanda Dice
Baltimore Talent Development High School (#428)
1500 Harlem Park Ave.
Baltimore MD 21217
aedice@yahoo.com
Mentored by Dr. Volker Mai, University of Maryland at Baltimore,
Epidemiology and Preventive Medicine
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Table of Contents

Teacher Section

Overview

In the intestines, humans depend on certain bacteria to digest fiber for them and maintain general gastrointestinal health. When these beneficial bacteria are fed the proper things they thrive and crowd out the other bacteria. These bacteria are also known to promote the production of the immunoglobulin IgA. These immunoglobulins, or antibodies, work to increase the ability of the immune system to fight off antigens.

Through this curriculum students will understand how the immune system works, see how bacteria play a role in the digestive system, and how food can affect the health of the immune system. Through the laboratory exercises, students will see how antibodies interact with antigens and how different foods are digested.

This series of lessons should be taught along with the immune system unit in a biology or health class. Students will be studying how the bacteria in the gut can affect overall health. They will use all of the information they gained from these lessons to create a diet plan for someone who has Crohn's disease and is trying to increase the health of the digestive system with diet.

Science Background

Immunoglobulins and Antibodies:

Immunoglobulins and antibodies are interchangeable terms. Immunoglobulins are the major class of proteins that antibodies belong to based on their overall protein structure.

Immunoglobulin: large class of glycoproteins that constitute the antibodies produced in response to antigenic stimuli.

Antibodies: Immunoglobulin multichain glycoproteins synthesized by B-cells and plasma cells (memory cells) in response to the introduction of foreign substances.

There are different immunoglobulin (Ig) classes (IgA, IgG, IgM, IgE, and IgD) that will produce different antibodies, each with specialized functions (due in part to structure) and specificity for an antigen.

Antigen-Antibody Interaction: The Ouchterlony Procedure:

Antibodies and antigens form complexes that precipitate, making it possible to assay antigen-antibody interactions. The objective of this experiment is to give students a visualization of this interaction. They will see three different types of reactions; a reaction of identity, a reaction of partial identity and a reaction of non identity.

This technique which uses double diffusion in two dimensions was invented by the Swedish scientist, Ouchterlony. In this procedure, solutions of antigens and antibodies are placed in separate wells cut in an agarose plate. The two will diffuse towards each other and begin to precipitate where they meet at equal proportions. Homologous antibody and antigen pairs will combine and form

a single precipitation line. Antigens will act independently of each other when two are present. The number of antigen-antibody systems present can be determined by counting the precipitation bands.

Reaction of Identity – When an antigen is placed in two adjacent wells and the homologous antibody is placed in the center of them, two precipitation lines will form. Where these two lines cross, they will fuse together. (see diagram in anticipated results section)

Reaction of Partial Identity – When an antigen is in one well, the homologous antibody is in the center well and the antigen in the adjacent well is a cross-reacting agent, the precipitation lines will fuse. There will also be a spur of the precipitation line that continues past the intersection. The spur always points to the homologous antigen. This happens because the homologous antigen contains determinants recognized by the homologous antibody which are not present on the cross-reacting antigen. Since these non-cross-reacting antibodies often compose only a fraction of the total antibody involved in the homologous precipitin reaction, the spur is usually less dense than the precipitin band from which it projects. (see diagram in anticipated results section)

Reaction of Non-Identity – When unrelated antigens are placed in adjacent wells and the center well is filled with antibodies of each antigen; the precipitin bands will form independently of each other and will cross. (see diagram in anticipated results section)

For an interactive demonstration go to
http://www.grisda.org/tstandish/teachers/presentations/
Click on the Ouchterlony Procedure link

Fiber and the Immune System:
IgA is the most abundant immunoglobulin in mucosal secretions in the intestines and plays an important role for the body's defense to protect against microbial infection. It is generally believed that Peyer's patches are the major site for IgA development in the gut. IgA production by B1 cells is T cell-independent and requires the presence of commensal microflora. The commensal microflora needed in the intestines to increase the amount of IgA are the beneficial bacteria that increase in population size when an individual eats a high fiber diet. Therefore, eating a diet high in fiber can lead to a healthier immune system.

Website:
http://www.askdrsears.com/html/4/t042500.asp

Terminology:
Probiotics
Ingesting live microorganisms that result in a health benefit to the host.

Prebiotics
non digestible food ingredient that beneficially affects the host by selectively stimulating the growth of good bacteria.

Inflammatory Bowel Disease
(IBD) is a group of disorders that cause the intestines to become inflamed. The inflammation is usually chronic and can have serious adverse affects on an individual's quality of life. Symptoms of IBD include abdominal cramps and pain, diarrhea, weight loss and bleeding from the intestines. Examples of IBD's are Crohn's disease and ulcerative colitis.

Bowel Disorders
Chronic problems with the bowels that usually include diarrhea, constipation and vomiting.

Websites on Inflammatory Bowel Diseases and Disorders
http://kidshealth.org/parent/medical/digestive/ibd.html
http://www.medicinenet.com/inflammatory_bowel_disease_intestinal_problems/article.htm
http://familydoctor.org/252.xml
http://www.healingwithnutrition.com/idisease/inflambowel/crohns.html

Learning Objectives

- Students will learn how the immune system works by participating in a game that explains the terminology and roles of different parts of the immune system
- Students will observe antibodies interacting with antigens through a laboratory that uses the Ouchterlony Procedure.
- Students will understand how the health of the digestive system, and more specifically the health of the intestinal microflora, can affect the effectiveness of the immune system. Students will learn about probiotics and prebiotics through lecture and independent research.
- Students will observe that the stomach does not digest fiber through a digestion simulation laboratory.
- Students will research and analyze different types of intestinal diseases and disorders.
- Students will apply their knowledge by using all of the data they have collected to create an immune system boosting diet plan for a patient that has Crohn's disease.

Time Requirements

For double class periods, about 5 days will be needed to complete these lessons.

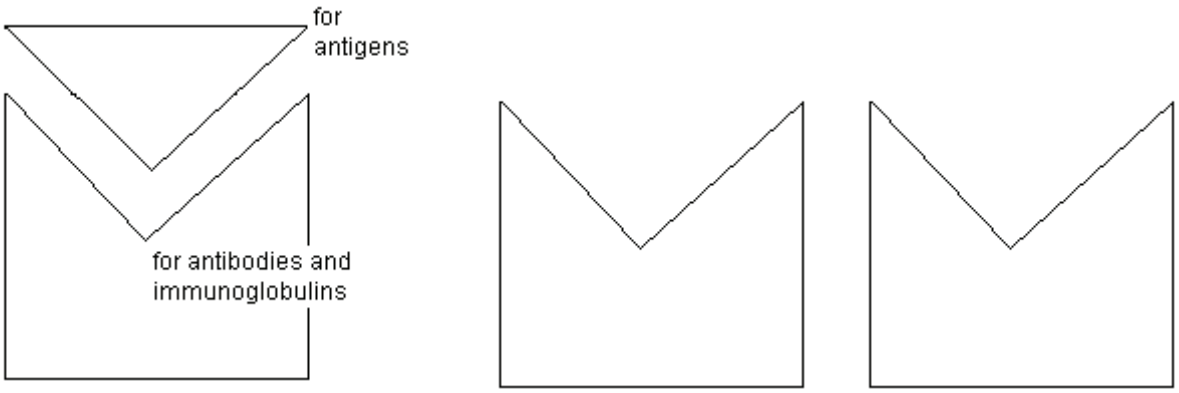
- Introduce terminology and play immune system game – 1day
- Set up Antigen-Antibody Interaction Lab and Digestion Simulation Lab – 1 day
- Analyzing results of both labs and drawing conclusions– 1 day

- Researching inflammatory bowel diseases and disorders – 1 day
- Creating a diet plan to boost immune system – 1 day

For single class periods 10 days will be needed, double all time requirements.

Advance Preparation

- Prepare for Immune System Game:
    1. Draw immune system diagram on board with two deflated balloons where the lymph nodes are
    2. Have 1 or 2 students in mind from each class that have good organizational skills and can learn information quickly. These students will be your narrators for the game.
    3. Create immune system cards. Make even amounts of antigen, antibody, immunoglobulin, macrophage, B cell and T cell cards.
    4. Make antigen and antibody/immunoglobulin receptors: Cut thick paper into around 6" squares. Cut out of the squares different shapes for the antigens to hold in their hands to model their receptors. Save the remaining piece of the square to be the receptors for the antibodies and immunoglobulins. You will need more for this group, so make some repeat receptors of a simple shape. (see below)



Extra receptors for larger antibody/immunoglobulin group

- Prepare for antigen-antibody interaction lab:

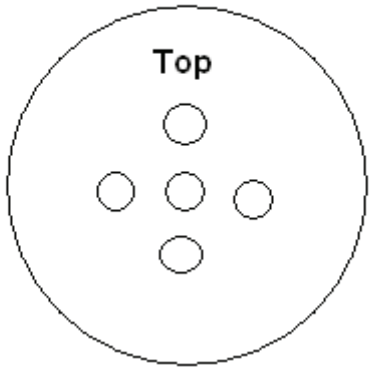
    Prepare the Agarose and pour Ouchterlony Plates

    1. In a 500 ml Erlenmeyer flask add 3g of powdered buffer to 225ml of distilled water. Swirl the flask to dissolve the powder.
    2. Add 3g of agarose to the flask. Swirl to dissolve any large clumps.
    3. Mark the flask with a wax pencil at the level of the liquid.
    4. Cover the flask with tin foil and boil over a hot plate or Bunsen burner. A microwave can also be used (no foil). Boil the mixture until all of the gelatinous agarose is dissolved. Swirl occasionally. The final solution should be clear with no particles of agarose in it.
    5. If the solution has evaporated during the heating process, add distilled water until it fills to the original volume at the marked line.

6. Cool the agarose solution to 55 degrees C in a waterbath. Swirl to promote even distribution of heat.
7. Pour 4 plates for each group: Use a 5ml or 10ml pipette to transfer 5ml of agarose in to each plate. Rotate the plate to ensure that the bottom is covered.
8. If there are bubbles in the agarose, they can be removed by passing a flame across the surface of the media.
9. Allow the agarose to solidify. This will take about 10 minutes. The gel will appear slightly opaque.
10. The plates can be stored for up to two weeks. Wrap with plastic wrap and store inverted in a refrigerator.

Cut Wells: (students may also do this as part of their lab)

1. Make 10 sample loading plates. These plates should contain 10 or more wells for students to practice loading the solution. These wells can also help students practice cutting the wells themselves.
2. Make 30 experiment plates. These plates should have wells cut to match the template below:



3. Label the practice plates P with marker
4. Label 10 experiment plates #1 with marker
5. Label 10 experiment plates #2 with marker
6. Label 10 experiment plates #3 with marker

Preparation of the Incubation Chamber:
(prepare the day of the lab)
Line the bottom of a plastic or glass container with several paper towels. Soak the paper towels with distilled water. There should not be a layer of water above the paper towels. Cover the entire chamber in plastic wrap.

Prepare antibodies and antigens:

1. label 10 microtest tubes A
2. label 10 microtest tubes B

3. label 10 microtest tubes C
4. label 10 microtest tubes D
5. Add 100 microliters of animal anti-serum sample into each test tube A
6. Add 200 microliters of animal serum sample into each test tube B
7. Add 150 microliters of alburnin antigen sample into each test tube C
8. Add 80 microliters of IgG antigen sample into each test tube D
9. Make 10 groups, each containing a test tube labeled A, B, C and D.

- Prepare for Digestion Lab:
    1. Boil eggs and cut the egg whites into approximately equal sized cubes.
    2. Cut broccoli stems into approximately equal sized cubes.
    3. Prepare 2% pepsin and trypsin solutions. Mix 4g of powder for every 200 ml of water.
    4. Set up a demonstration of how test tubes should be labeled in the front of the room

- Prepare Label Packet
  About a week before starting this lesson, tell students that they need to bring in 3 food labels by the end of the week. You should gather some as well. You will need to photocopy the labels and make 30 packets. The idea is that students will look through this packet at the end of this curriculum and identify foods high in fiber. They will be making a high fiber diet plan using this information. You should include some very healthy high fiber labels so the students have plenty to choose from. Anticipate the labels that the students bring in to represent less healthy foods.

Materials and Equipment

Ouchterlony Procedure (for a class of 30 – 10 groups)

2 ml Animal anti-serum sample (contains antibodies)
2.5 ml Animal serum sample (antigen)
2.5 ml Alburnin antigen sample
1.5 ml IgG antigen sample
3g powdered buffer
3g agarose
40 transfer pipets
40 petri plates
10 well cutters (plastic straws)
1 template for cutting wells (see advance preparation section)
40 microtest tubes
500 ml flask or beaker
Micropipette and tips (optional)

Plastic or Pyrex container
Plastic wrap
Distilled water
5ml or 10ml pipets
Marking pen
Measuring spatula or toothpicks
Heat plate, Bunsen burner or microwave
Paper towels
Waterbath (55 degrees C)

Fiber Digestion Lab (for class of 30 – 15 groups)
60 test tubes
Wax pencils
15 test tube racks
15 balances or scales
12 boiled eggs
Box of Bran Flakes or other high fiber cereal
Broccoli
15 graduated cylinders
150 ml of 2% pepsin solution
150 ml of 2% trypsin solution
Distilled water

Student Prior Knowledge

Before starting these lessons, students should understand the structure and function of antibodies and antigens as well as how the body reacts to foreign invaders. They should have a strong background in how both the immune system and digestive system work. In particular, they should be able to explain what happens to food as it moves through the intestines and how the body gets nutrients from food. They should also be familiar with the basic foundations of nutrition.

Students should be able to use pipets, measure accurately and be able to take detailed notes from observations.

Student Expectations

- Complete antigen-antibody interaction laboratory and turn in results and conclusions
- Complete bacteria and fiber laboratory and turn in lab sheet
- Collect labels for foods that help promote a healthy immune system
- Complete independent research on bowel diseases and disorders
- Create a diet plan that helps promote a healthy digestive system and immune system for someone who is HIV positive

Anticipated Results

Antibody-Antigen Interaction Lab:

Students should see the following results in their three plates:

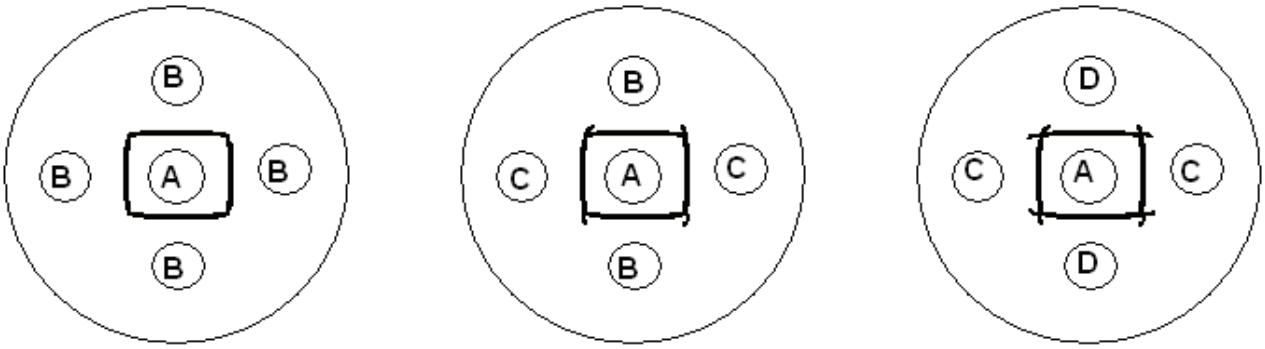


Plate 1 Plate 2 Plate 3

Plate 1 shows Reaction of Identity.
Plate 2 shows Reaction of Partial Identity
Plate 3 shows Reaction of Non-Identity

Absence of precipitation lines may be due to uneven pipetting of samples into the wells or incorrect distances between wells.

Digestion Simulation Lab:

Test tube 2 (egg) should look very different from test tube 1 (control) after 24 hours.
Test tube 4 (bran flakes) should look very similar to test tube 3 (control) after 24 hours.
Test tube 6 (unknown-broccoli) should look very similar to test tube 5 (control) after 24 hours.
Students should realize that eggs do not have fiber in them; therefore they are easily digested by acids in the stomach. They should recognize that the bran flakes are high in fiber and cannot be digested by stomach acids. Fiber must be broken down by bacteria in the intestines. The unknown food (broccoli) is high in fiber and students should come to this conclusion based on their observations.

Lesson Planning/Classroom Discussion

Day 1

Review of immune system and Disease Prevention lab:

Introduce terms:

1. Antigen (foreign substance in body)
2. Macrophage cell (engulfs antigens and presents antigen to B or T cells) antigen presenting cells (apc)
3. B cells (make several different antibodies. If an antibody binds or fits with an antigen it signals one of several responses to destroy the antigen)

4. T cells (mature in the thymus, when in contact with an antigen it either alerts B cells to make antibodies, activates other T cells or becomes a killer cell and destroys antigen)
5. Immunoglobulins (any of a group of large glycoproteins that are secreted by plasma cells and that function as antibodies in the immune response by binding with specific antigens. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM.)

Play Immune System Game:

- Hand out the "Immune System Game" worksheet
- Have students you picked to be the narrators go sit in front of the board by the lymph nodes.
- Every student draws a card and becomes an antigen, antibody, immunoglobulin, macrophage, B cell or T cell. (you may want to have students tape the card to their shirts so it is clear what everyone's role is during the game)
- Designate a table for immunoglobulins and antibodies. Send the appropriate students there. (this table should be next to the "B cells" table)
- Designate tables for T cells and B cells and send the appropriate students to these tables.
- Have macrophages wandering around the room as if they were in the blood stream
- Describe the classroom as being a human body. Antigens should group up by the door. Teacher should direct them to enter one at a time, just as antigens enter our bodies.
- Give antigens their "receptor" shapes to hold in their hands
- Give the antibodies and immunoglobulins their "receptor" shapes to hold. (these will indicate which one matches up with which antigen)
- Go over the terminology at the top of the worksheet. Have students raise hands and brainstorm a few ideas about different scenarios that could play out when an antigen enters the body.
- Students should use new terms to answer questions 1-4 on worksheet
- Have narrators create one scenario and direct the different players to do different things according to the scenario.
- You may want to run through each scenario 2-3 times. After each scenario, students should record what happened on worksheet.
- Do as many scenarios as time will allow.

Assessment

- Ask students what the roles of each of the parts of the immune system.
- Revisit the definitions and questions on the worksheet.
- Have students explain what was done that day in their own words.

Day 2
Begin Experiments

Antigen-Antibody Interaction: The Ouchterlony Procedure

- Explain to students that when antibodies react with antigens, a visible precipitate forms. This is what they will be looking for when doing this procedure.
- Teach students about the three results they should expect to see; the reaction of identity, the reaction of partial identity and the reaction of non-identity. Explain to them the conditions in which each of these three results would occur. (see background knowledge)
- Demonstrate steps of the lab in the front of the room (Petri dishes could be placed on overhead projector to enlarge it)
- When doing the lab, relate different activities back to the game the day before. Discuss major points before beginning the lab.

Digestion of Fiber and Protein Lab

- Students will begin the Digestion of Fiber and Protein Lab. They will be able to see results on day 3.
- They should write their hypothesis before beginning the lab.
- The unknown food for this lab is broccoli which is a food high in fiber. The idea is that the pepsin and trypsin will not digest the broccoli and the students will conclude that it is high in fiber.
- Students should follow directions on their lab sheets. They should use the data table provided to record results.
- Demonstrate steps in the front of the room.
- While students are waiting to make first observation (at 5 min. mark), they can clean up supplies
- While students are waiting to make second observation (at 15 min. mark), they should revisit their hypothesis and see if the first observation agrees with it. Students should write a few sentences on the back of their worksheet describing if they still agree with their hypothesis and why or why not.

Assessment

- Observe students for participation during labs
- Students will turn in lab results and conclusions

Safety Notes for Labs:
Go over the following with your students (you may want to photocopy this)

1. Know where the safety items in the room are. These should include the eye wash, first-aid kit and fire extinguisher.
2. Wear a laboratory coat or apron and protective glasses or goggles for all laboratory work. Wear shoes (rather than sandals) and tie back loose hair.

3. Clear your bench top of all unnecessary materials such as books and clothing before starting your work.
4. Check chemical labels twice to make sure you have the correct substance.
5. You may be asked to transfer some laboratory chemicals from a common bottle or jar to your own test tube or beaker. **DO NOT** return any excess material to its original container unless authorized by your teacher.
6. Never taste laboratory materials. Gum, food, or drinks should not be brought into the laboratory. If you are instructed to smell something, do so by fanning some of the vapor toward your nose. Do not place your nose near the opening of the container.
7. Never look directly down into a test tube; view the contents from the side. Never point the open end of a test toward yourself or your neighbor.
8. Any laboratory accident, however small, should be reported immediately to your teacher.
9. In case of a chemical spill on your skin or clothing rinse the affected area with plenty of water. If the eyes are affected water-washing must begin immediately and continue for 10 to 15 minutes or until professional assistance is obtained.
10. Minor skin burns should be placed under cold, running water.
11. When discarding used chemicals, carefully follow the instructions provided.
12. Return equipment, chemicals, aprons, and protective glasses to their designated locations.

<u>Day 3</u>
Finding Results and Discussion

Antibody-Antigen Interaction Lab
- Students spend a few minutes looking at their plates and drawing what they see on the diagrams on their lab paper.
- Students should answer questions at the end of lab. Discuss answers with class.

Digestion of Fiber and Protein Lab
- Students spend a few minutes making their final observations and recording results on data table.
- Fiber and Bacteria: Activate prior knowledge on bacteria (think back to environmental science and bacteria's role as decomposers). Tell students to think of the intestines as just a different ecosystem. Ask students to make a hypothesis about what would happen if bacteria were added to the indigestible fiber.
- Discuss the idea that eating foods high in fiber helps promote healthy bacteria in the intestines.

- Ask a few students to give their results. Ask them why they think the Bran Flakes did not break down very much. Explain that fiber cannot be digested in the stomach, but depends on bacteria in the intestines to break it down.
- Discuss with the class the following ideas: When a person eats a lot of fiber, this gives the beneficial bacteria plenty to eat, so their populations grow (prebiotics) Having a high count of beneficial bacteria in the intestines promotes the level of the immunoglobulin IgA. This, in turn, promotes the health of the immune system by increasing its antigen fighting potential
- Students should now answer questions on the same piece of paper as their hypothesis. They should each turn in this piece of paper and their data table at the end of class or finish for homework.

Day 4

Diet Plan Preparation and Background Information

- Good vs. Bad Bacteria – ask students what they think good bacteria do for humans in the digestive track (digest fiber…refer back to lab). Why is this helpful for humans? Bad bacteria cause food not to be digested properly and diarrhea. Good bacteria take over the bad bacteria if they are fed the proper things, like fiber.
- Do the following activity with some students in front of room to demonstrate good bacteria crowding out bad bacteria: Create a section of an intestine on the floor in the front of the room with tape. Make it big enough for about 15 students to stand in it. Make fiber cards and junk food cards and shuffle them together in a pile. Have 1/3 of the class be “bad bacteria” and 2/3 of the class be “good bacteria”. Turn over cards one at a time. For every fiber card 2 good bacteria can enter intestine and for every junk food card, one bad bacterium enters the intestine. When the intestine starts to fill up, the bad bacteria are crowded out
- Remind students that the good bacteria promote higher levels of the immunoglobulin IgA, which helps promote overall health.
- Discuss with students that there are many diseases and disorders of the bowel that are due to imbalances in the gut bacteria. When a person has one of these diseases or disorders, the ability to absorb nutrients from food is compromised as well as the immune systems ability to fight infection.
- Students will do independent research on the internet to learn about different intestinal diseases and disorders. They will fill in the chart in the student section and use it as their guide.
- Encourage students to document the websites where they got information from
- Students read the article, “Fiber-boosted formula for piglets shows promise in battle against infant diarrhea” (in student section) for homework and create a 3 paragraph summary with the main points of the article.
- Before leaving class, ask students, “Why are we talking about diet while studying the immune system”. Discuss responses. Go over questions associated with the article.

Assessment – have students report their findings at the end of class and check homework activity

Sample Intestinal Diseases and Disorders Chart:

Disease	Cause	Symptoms
Crohn's Disease	Cause not proven. It is thought that the body's immune System reacts to bacteria or viruses. Immune system activated when there is not a foreign invader.	Intestinal wall thickens and scars. Blockage of the intestine. Intestines become sore and swollen. Sores and ulcers on intestines. Food is not absorbed properly. Can affect any part of the digestive system.
Colitis	Same as Crohn's disease.	Inflames inner lining of the colon and rectum. Abdominal pain, bloody diarrhea, fatigue, weight loss, loss of appetite, fever and nausea.
Irritable Bowel Syndrome (IBS)	Cause is not proven. Individuals are thought to have sensitive intestines and colon. Certain foods and stress. Immune system may be involved.	Need to defecate immediately Spasmodic colon Diarrhea and constipation Abdominal pain
Gastroenteritis	Bacteria and bacterial toxins. Parasites, viruses, some chemicals and drugs	Abdominal cramps Diarrhea Vomiting
Constipation	Not enough fiber or liquids in diet. Not enough exercise. Certain medications. IBS	Inability to defecate
Diarrhea	Bacteria or viral infections. Parasites Certain foods or medications.	Loose, watery stools occurring several times a day. Dehydration

Main ideas in homework article:

- The ongoing research at the University of Illinois at Urbana-Champaign is targeting a $2 billion-a-year, sometimes fatal problem – acute diarrhea – that, according to the Centers for Disease Control and Prevention, accounts for

about 9 percent of all hospitalizations of children under age 5 in the United States annually

- Our hope is that infant formulas can be enhanced to provide much of the same activity that a mother's milk will do
- The gastrointestinal tract and general development of neonatal piglets are similar to that of human infants born about eight weeks early.
- A control group received a standard sow-milk replacement formula. A second group got formula supplemented with non-fermentable fiber (methylcellulose). The other two groups, respectively, received formulas containing soy polysaccharide (a moderately fermentable fiber) and fructo-oligosaccharides (a highly fermentable fiber).
- After seven days, the piglets were infected with the salmonella strain that commonly occurs in infants
- there was a reduced incidence of diarrhea, and the activity level was maintained for those on the higher fiber diets," Tappenden said. "The control animals and those that were fed the non-fermentable fiber developed severe diarrhea and became very lethargic. What that tells us is that it is not just fiber that is important, but fiber quality is very important and that we need a fermentable fiber."
- "By our results, we could see that adding dietary fiber does not seem to alter the development of a newborn's intestinal tract," she said. "It made no difference to the healthy group. The presence of the fiber did help in an infectious state, however. Thus it may be that all newborns could consume the fiber without the worry of negative consequences on their development."

<u>Day 5</u>

Creating a Diet Plan

- Students will pretend they are doctors prescribing a diet plan for a patient with Crohn's disease. The goal is to create a diet plan that promotes the health of the immune system and digestive system as much as possible.
- Review homework from night before. Have a few students volunteer to read one of their paragraphs.
- Put the following questions on the board. Students should answer them underneath their homework paragraphs:
    1. Summarize the procedure used for this experiment.
    2. What were the results?
    3. Use the results of this experiment to make an inference about how human infant formula could be improved.
- Discuss answers and collect all work.
- Students should look through photocopied packets of food labels and identify high fiber foods on their worksheet.
- They should use these foods as well as do some independent research to create a menu for a breakfast lunch and dinner for their "patient".
- They should describe how the foods they have chosen are healthy choices for the digestive and immune systems.
- The role of bacteria in the gut should also be discussed.

- At the end of class have students look at the diet plan they created and list any foods from their plan that they eat themselves. Ask students how their diet compares to the one they just created.
- For homework, have students write down everything they eat in one day and have them make suggestions for themselves about how their diet could be healthier.

Student Section

Name_____
Date_____

Rationale Page---Why are we talking about diet when we are learning about the immune system?

We all know that some foods are considered good for us and some foods are not. What you may not know is that there are certain foods that not only contain importance nutrients, but also have the ability to strengthen your immune system. You will be taking a closer look at what these foods are and the positive affect they have on the body through two labs, independent research and a project. You will also get the opportunity to see how your diet measures up to what is considered healthy.

Introduction

In the intestines, humans depend on certain bacteria to digest fiber for them and maintain general gastrointestinal health. When these beneficial bacteria are fed the proper things they thrive and crowd out the other bacteria. These bacteria are also known to promote the production of the immunoglobulin IgA. These immunoglobulins, or antibodies, work to increase the ability of the immune system to fight off antigens. Therefore, eating foods high in fiber can actually increase the strength of the immune system. This can be especially important for individuals with a bowel disorder or disease.

Through the next few days you will understand how the immune system works, observe how bacteria play a role in the digestive system and see how food can affect the health of the immune system. Through the laboratory exercises you will see how antibodies interact with antigens and how different foods are digested. You will use all of the information you gained from these lessons to create a diet plan for someone who has Crohn's disease and is trying to increase the health of the digestive system with diet.

Background Information

<u>Immunoglobulins and Antibodies:</u>

Immunoglobulins and antibodies are interchangeable terms. Immunoglobulins are the major class of proteins that antibodies belong to based on their overall protein structure.

Immunoglobulin: large class of glycoproteins that constitute the antibodies produced in response to antigenic stimuli.

Antibodies: Immunoglobulin multi-chain glycoproteins synthesized by B-cells and plasma cells (memory cells) in response to the introduction of foreign substances.

There are different immunoglobulin (Ig) classes (IgA, IgG, IgM, IgE, and IgD) that will produce different antibodies, each with specialized functions (due in part to structure) and specificity for an antigen.

Fiber and the Immune System:
IgA is abundant in the intestines and plays an important role for the body defense to protect against infections. IgA production requires the presence of beneficial bacteria. The beneficial bacteria increase in population size when an individual eats a high fiber diet. Therefore, eating a diet high in fiber can lead to a healthier immune system.

Terminology:
Probiotics
Ingesting live microorganisms that result in a health benefit to the host.

Prebiotics
non digestible food ingredient that beneficially affects the host by selectively stimulating the growth of good bacteria.

Inflammatory Bowel Disease
(IBD) is a group of disorders that cause the intestines to become inflamed. The inflammation is usually chronic and can have serious adverse affects on an individual's quality of life. Symptoms of IBD include abdominal cramps and pain, diarrhea, weight loss and bleeding from the intestines. Examples of IBD's are Crohn's disease and ulcerative colitis.

Bowel Disorders
Chronic problems with the bowels that usually include diarrhea, constipation and vomiting.

Name_____
Date_____

Immune System Game

Review the list of new terminology below. Use this information to answer questions 1-4.

Antigen (foreign substance in body)
Macrophage cell (engulfs antigens and presents antigen to B or T cells) antigen presenting cells (apc)
B cells (make several different antibodies. If an antibody binds or fits with an antigen it signals one of several responses to destroy the antigen)
T cells (mature in the thymus, when in contact with an antigen it either alerts B cells to make antibodies, activates other T cells or becomes a killer cell and destroys antigen)
Immunoglobulins (any of a group of large glycoproteins that are secreted by plasma cells and that function as antibodies in the immune response by binding with specific antigens. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM.)

1. What is the job of the macrophage?

2. Why do you think lymph nodes become swollen when there is an infection in the body?

3. What are the two roles the T cells can play?

4. How do antibodies and immunoglobulins know if there is an antigen present in the body?

Use the space below and on the back of this page to record what happened during different scenarios of immune system game:

Scenario 1

Scenario 2

Scenario 3

Scenario 4

Name_____
Date _____

Antigen-Antibody Interaction Observation Lab

Description

In this lab you will see antibodies binding to antigens. When the two interact, they will form precipitation lines in the agarose. Different line formations will appear in the agarose depending on which types of antigens are added to the wells surrounding the antibody. You will use one plate to practice loading the wells and three plates to run the experiment.

Materials

Goggles and gloves for each person
marker
1 practice loading plate (labeled P)
3 experiment plates (labeled 1, 2 and 3)
1 microtest tube labeled A (contains antibodies)
1 microtest tube labeled B (contains animal antigen)
1 microtest tube labeled C (contains alburnin antigen)
1 microtest tube labeled D (contains IgG antigen)
4 pipets

Procedure

1. Practice loading wells. Using a pipet, practice loading water into the wells on the practice loading plate. Here are some tips for loading the wells: squeeze the pipet stem, not the bulb, to slowly draw a portion of the water (or sample) up into the pipet. The water (or sample) should remain in the lower portion of the pipet. Place the pipet just over, not inside, the sample well. Squeeze the pipet stem with even pressure so that the liquid does not move up the pipet. Slowly squeeze the pipet bulb to eject two drops of liquid. The well should appear full, but do not over fill. The liquid should not be present on the agarose surface.
2. Place 2 drops (30 microliters) of sample A (antibodies) in the center well of all three plates.
3. Add samples B, C and D (antigens) to the plates. Use the diagrams below when adding each antigen. Be sure to use a clean pipet for each antigen. The pipets may be used more than once as long as it is not contaminated by touching another antigen. Be careful when filling the wells. Remember not to over fill them.

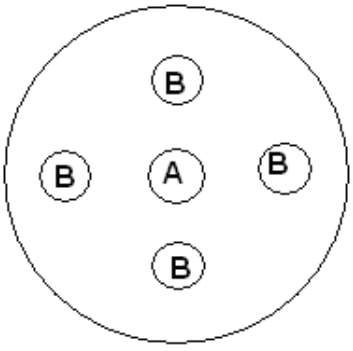


Plate 1

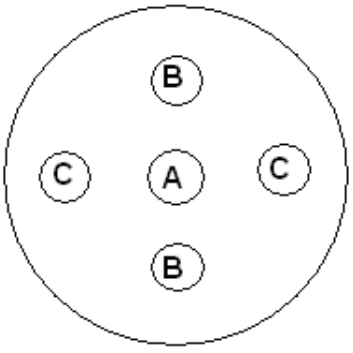


Plate 2

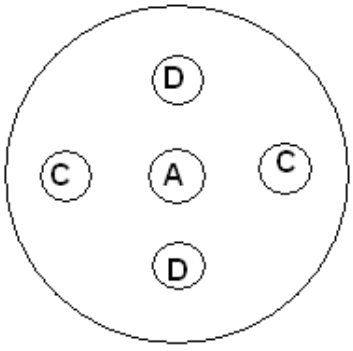


Plate 3

****put a mark with marker on the outside lip of the plate above the top sample. This will let you know where the top of the plate is when looking at your results.

4. Put lids on the plates. Put your group number on each of the three lids with the wax pencil.
5. Place plates in the incubation chamber on top of the wet paper towels. Once all plates are in, the chamber will be covered. Allow plates to incubate for 24-48 hours.
6. Take plates out of the incubation chamber. Hold each plate up towards the overhead lights. You should see opaque white arcs in each plate where the antibody and antigen precipitated.
7. Draw your results on the plate diagrams above.

Conclusions

1. Using your notes from yesterday, describe what happened in each plate and why it happened.
2. In plate 2, which antigen is the cross-reacting antigen? How could you tell?
3. Compare your results with the rest of the class. Did they turn out similar or different? Explain why your group or another group may have gotten unexpected results? What could be some of the causes for this?

Name_____
Date _____

Observing Digestion of Foods without Fiber vs. Foods High in Fiber and Determining the Fiber content of an Unknown

Description

In this lab, you will observe how stomach acids change food. You will test the affect of pepsin and trypsin on egg whites, a food low in fiber and on Bran Flakes cereal, a food high in fiber. You will also test an unknown food item to determine if that food is low or high in fiber.

Hypothesis

Write a prediction about how you think the results of the first part of this experiment will turn out. Revisit what you have already learned about fiber and digestion to assist you in this. You must be able to back up your prediction with solid facts.

Materials

Read through the list of materials below. Make sure you and your partner have the following things. Check off each item as you identify them:

- 2 pairs of gloves and goggles
- 2 aprons
- Wax pencil
- 6 test tubes
- 1 test tube rack
- 2 pieces of boiled egg white
- 2 pieces of Bran Flakes cereal
- 2 pieces of unknown food
- 1 graduated cylinder
- 2% pepsin solution
- 2% trypsin solution
- distilled water

Procedure

1. Label test tubes 1-6 with the wax pencil
2. Items tested in each test tube are as follows: (look at demonstration in front of the room)

Test Tube #1 Non Fiber Digestion Control	**Test Tube #4 Fiber Digestion Experiment**
Test Tube #2 Non Fiber Digestion Experiment	**Test Tube #5 Unknown Food Digestion Control**
Test Tube #3 Fiber Digestion Control	**Test Tube #6 Unknown Food Digestion Experiment**

3. Put on gloves, apron and goggles
4. Add a piece of egg white to test tubes 1 and 2
5. Add a piece of bran flakes to test tubes 3 and 4
6. Add a piece of unknown food to test tubes 5 and 6
7. Add 5ml of distilled water to test tubes 1, 3 and 5
8. Add 2.5ml of pepsin solution to test tubes 2, 4 and 6
9. Add 2.5 ml of trypsin solution to test tubes 2, 4 and 6
10. Gently swirl each test tube for 10 seconds each
11. Observe the test tubes for 5 minutes and record results in data tables
12. Let sit overnight
13. Record observations in data tables

Data Tables
(Fiber vs. Non-Fiber Digestion)

Name _____
Date_____

Observations

Time	Egg White (non fiber food)	Bran Flakes (high fiber food)	Unknown Food
5 min			
15 min			
24 hr.			

Questions

1. Compare test tubes 1 and 2. Did the egg whites change much in 24 hours? Explain why this happened.
2. Compare test tubes 3 and 4. Did the bran flakes change much in 24 hours? Explain why this happened.
3. Compare test tubes 5 and 6. Did the unknown food change much in 24 hours? Do you think this food is high or low in fiber? Explain your answer.
4. If high fiber foods are not digested by stomach acids, how does the body break them down?
5. How does fiber digestion help promote the health of the immune system?
6. What is the purpose of the control?
7. Do your results support what you wrote as the hypothesis? Did your experiment turn out the way you expected it to? What elements in the experiment could have affected the outcome?

Name_____
Date_____

Use the internet to research the disorders and diseases below. Use 2 – 3 different websites to gather information from for each.

Intestinal Diseases and Disorders Chart

Disease	Cause	Symptoms	Websites Visited
Crohn's Disease			
Colitis			
Irritable Bowel Syndrome (IBS)			

Gastro-enteritis			
Constipa-tion			
Diarrhea			

Name_____

Homework: Read the following article. Find 3 main topics of the article. Write a paragraph about each one describing what the topic is and why it is important. Be ready to discuss this article during class tomorrow.

Inside Illinois

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Fiber-boosted formula for piglets shows promise in battle against infant diarrhea

Jim Barlow, Life Sciences Editor

6/2/03

CHAMPAIGN, Ill. — Adding fiber to the diet for bowel health is standard advice for adults. Such wisdom also may benefit babies, say researchers who are testing the impact of fiber added to milk-replacement formulas of newborn piglets.

In the June issue of the Journal of Nutrition, the researchers reported that piglets that consumed formula with moderate levels of fermentable fiber tolerated an induced infection of Salmonella typhimurium much better than those fed a plain control formula or one with a non-fermentable fiber.

The ongoing research at the University of Illinois at Urbana-Champaign is targeting a $2 billion-a-year, sometimes fatal problem – acute diarrhea – that, according to the Centers for Disease Control and Prevention, accounts for about 9 percent of all hospitalizations of children under age 5 in the United States annually. While experts encourage mothers to breast feed their infants to stimulate natural protection against a multitude of diseases, the vast majority of infants are fed infant formulas.

"We know that breast milk is important because it sets up microbiota in the colon and fights off infections," said Kelly Tappenden, professor of food science and human nutrition and principal investigator of the research. "Infants are most susceptible to diarrhea diseases compared to other segments of the population. Many such diseases peak in the first year of life. We are interested in trying to prevent diarrhea-related diseases in babies. Our hope is that infant formulas can be enhanced to provide much of the same activity that a mother's milk will do."

Tappenden and colleagues divided 48 two-day-old piglets into four groups fed in equal amounts every 12 hours. The gastrointestinal tract and general development of neonatal piglets are similar to that of human infants born about eight weeks early.

The more rapid growth rate of the piglets allows researchers to observe changes at a rapid pace.

A control group received a standard sow-milk replacement formula. A second group got formula supplemented with non-fermentable fiber (methylcellusose). The other two groups, respectively, received formulas containing soy polysaccharide (a moderately fermentable fiber) and fructo-oligosaccharides (a

highly fermentable fiber).

The fiber sources are among multiple possibilities and were chosen to test a broad range of levels, Tappenden said. The soy fiber is different than the soy protein that is used in soy-based formulas for babies that are allergic to cow's milk.

After seven days, the piglets were infected with the salmonella strain that commonly occurs in infants. The piglets' activity and signs of illness were monitored for seven more days, after which the piglets' small intestines and colons were evaluated.

"There was no change in body temperature among the piglets, but there was a reduced incidence of diarrhea, and the activity level was maintained for those on the higher fiber diets," Tappenden said. "The control animals and those that were fed the non-fermentable fiber developed severe diarrhea and became very lethargic. What that tells us is that it is not just fiber that is important, but fiber quality is very important and that we need a fermentable fiber."

In the experimental piglets, there were positive changes in the transport of nutrients. However, Tappenden said that her team might have waited too long into the recovery process to do the analysis, preventing an accurate determination of what the changes were and why they occurred.

"So now we are looking at the first 48 hours after infection," she said. "We know that fermentable fiber is doing something positive, but we still cannot say why. These new tests may help us answer that by looking at the conditions at the peak of infection."

Fibers are fermented in the body into short-chain fatty acids, which are short lipid molecules that are thought to be good for the digestive tract, providing increased intestinal structure and function, as well as promoting resistance against invading pathogens.

These pre-clinical results are encouraging but far from leading to changes in infant formulas, Tappenden said. "By our results, we could see that adding dietary fiber does not seem to alter the development of a newborn's intestinal tract," she said. "It made no difference to the healthy group. The presence of the fiber did help in an infectious state, however. Thus it may be that all newborns could consume the fiber without the worry of negative consequences on their development."

The Illinois Council on Food and Agricultural Research funded the project.

Authors of the study were Tappenden; Nancy J. Correa-Matos, a doctoral student in the department of food science and human nutrition; Sharon M. Donovan, holder of the Melissa M. Noel Chair in Nutrition and Health in the College of Agricultural, Consumer and Environmental Sciences and professor of pediatrics in the College of Medicine at Urbana-Champaign; Richard Isaacson, professor in the department of veterinary pathobiology in the College of Veterinary Medicine; and H. Rex. Gaskins and Bryan A. White, professors in the departments of animal sciences and veterinary pathobiology.

Name_____
Date_____

Creating a Diet Plan that Promotes the Health of the Immune System

I. Identifying foods that are high in fiber

A. Look through the photocopied packet and list foods below that are high in fiber. Be sure to list an equal amount of breakfast, lunch and dinner foods.

Food	Breakfast, Lunch Or Dinner?	Serving Size	Amount of Fiber Per Serving

B. Use the internet to research foods that are high in fiber. Record any information you find in the chart below. Include foods typically eaten for breakfast, lunch and dinner.

Food	Breakfast, Lunch Or Dinner?	Information

II. Create A Diet Plan

You will now pretend you are a doctor and use the information you gathered above to create a diet plan for your "patient" that has been diagnosed with Crohn's disease.

1. You will plan a breakfast, lunch and dinner for your patient on notebook paper. Make three sections, one for breakfast, lunch and dinner.
2. In each section, describe what the meal will consist of. With each food item tell how many servings and the fiber content. Make sure you are creating a daily diet plan that corresponds with the current food pyramid. Refer to a food pyramid guide as you work.

At the end of the diet plan, write 2-3 paragraphs explaining how the food items you picked for your patient to eat would help him or her maintain a healthy population of bacteria in the intestines and how this in turn would help promote a healthy immune system.