Lab 4 - Cells to Organisms

(June 2014)

Section 1- Molecules Essential to Cells

[2] Welcome to Lab 4. This is Pat Farris speaking and today I'll be introducing you to the things that make you an organism. We'll

start with the internal workings of cells and go through tissues, organs and organ systems. Let's get started.

[3] To begin, let's do a little review of the chemistry that will be essential to today's lab. Take a moment to think about all the chemicals that you take in on a daily basis, like oxygen, food and water. Where do these molecules go? What happens to them along the way? Do they ever come out again? It's a lot to think about!

[4] All the chemicals you take in eventually end up inside your cells. For example, your cells require a continuous supply of oxygen every minute of the day. They also demand feeding on a regular basis, and these cells need a way to unload the waste products they are generating. Now you've all just finished two labs on chemistry, so before we begin discussing how cells will obtain and use molecules, please answer the chemistry questions at the beginning of Section 1. Come back to the program when you've finished.

[5] For your list of chemicals essential to animal cells, I hope you included water and oxygen. For the other molecules, any type of carbohydrate, fat or protein would have been good answers as well. As for the other questions, I hope those were pretty simple review questions. Let's go on.

Section 2 - Membrane Structure

[6] Before we get to whole cells, we need to consider the boundary of all cells, the cell membrane, sometimes referred to as the plasma membrane. As I mentioned, ALL cells have cell membranes, so let's take a look at this universal structure.

[7] The first thing to consider is that the cell membrane is not just an inert bag that holds things in. It is a very dynamic structure. It's the cell membrane that determines if things get in or out of a cell to keep it alive and it's the cell membrane that allows communication between cells. For example, it's important for your immune system to recognize the cells which are yours, so if your body is invaded, the immune system can attack the interlopers without harming the cells it recognizes as you.

[8] The membrane is generally represented by the Fluid Mosaic Model. It's referred to as "fluid" because there's nothing really solid about it - it is a fluid with edges, as you'll see. To begin this structure, we need to review the structure of phosopholipids.

[9] Take a moment and recall the structure of a phosopholid. Remember the long tail represented a long non-polar chain of CH_2 groups and the small circle at the other end represents the polar section of the molecule. In other words, the two ends of the molecule act differently in response to water. Copy this diagram and label the hydrophobic and hydrophilic sections on your drawing.

[10] If we were to put these phospholipids into water, they would arrange themselves with the least amount of drama. What I mean by that is that the hydrophobic parts would get as far from the water as they could, and the hydrophilic parts would get as close to the water as they could, as shown in your lab book. This results in an arrangement called a phospholipid bilayer, which as you could probably guess, means there are two layers in which all the molecules have their preferred arrangement regarding water. Label the hydrophobic and philic areas of the bilayer as shown here.

[11] Well that's a very pleasant arrangement, but all we have so far is a boundary between two water-based environments. What we really need is the outer layer of a cell, which will require oxygen, water and food molecules to get in and for waste molecules to get out. This is where the "mosaic" part of the model comes in.

[12] In art, a "mosaic" is made up of thousands of little pieces of different colors that, when viewed from a distance, become a complete picture. The cell does the same thing with different organic components. A cell membrane is a collection of millions of phospholipids which have other organic molecules incorporated into the bilayer to accomplish the bigger job of moving molecules in and out of the cell.

[13] These additional components are listed here and in your lab book. Record the various roles of these molecules in the table. I'm sure you've heard of cholesterol before, but you may not have realized it is an important part of cell membranes, sometimes making up 25% of the membrane.

[14] And here are those molecules in place in a complete Fluid Mosaic Model. Please go back to the phospholipid bilayer drawing in Section 2 and make it a complete membrane by adding at least one protein, carbohydrate and a cholesterol molecule to your drawing, as shown here.

[15] If we were able to turn into microscopic people and walk up to a membrane and touch it, it would be like touching mercury. It's a firm fluid. The only thing holding the molecules in their proper orientation is their reaction to water. The proteins have R-groups that give them polar or non-polar parts, the carbohydrates stick out into the water environment where they are stable, and the cholesterol is a lipid, so it stays next to the phospholipid tails.

[16] Now let's take a closer look at just one type of protein. The three dimensional structure reveals that this one has a little channel that runs through it. Aha! So now we can see which of these membrane molecules will let stuff in and out of the cells. That's the job of the proteins.

[17] Now remember the proteins we looked at in last week's lab - the enzymes? What better molecule to incorporate into a membrane than one that can change chemicals as they enter the cell? The membrane proteins are often referred to as the "gate-keepers" of the cell, since they have control over any molecules moving through the membrane.

[18] Whether a molecule gets into a cell or not depends on its size, shape and electrical charge. Here are a variety of proteins that are incorporated into membranes and they all have unique roles in controlling what gets in and out of cells. Answer the last question in Section 2, then we'll be ready to go on to Section 3.

Section 3 - Organelles

[19] The term "organelle" means "little organ". This term refers to the little compartments in a cell that have certain jobs. Please record the definition of organelle in your lab book - organelles are membrane-bound structures within the cell, each with a specific function.

[20] And now that we've used the term cell, we should probably stop and define that too. Please record the significance of the cell in your lab book - cells are the smallest unit of life, each one carrying on the functions associated with life, such as metabolism, homeostasis, growth and reproduction.

[21] We will eventually look for organelles within cells, but there are two components that aren't generally obvious in a cell but are nevertheless very important. The first of these is the fluid matrix containing the organelles called cytoplasm. As we look at living cells, we may see the organelles seeming to float within this fluid. Record the definition in your book.

[22] Another component of the cell we generally can't see is the cytoskeleton, an intricate network of protein fibers that lend support and shape to a cell. We aren't equipped to do the specialized cell preparations required to see these structures, but I can show you one here on the screen. Pretty amazing to realize that most cells have a hidden scaffold, huh? Please record the definition in your book before we go on.

[23] There are two major cell types that we'll be comparing, and it will depend on whether or not the cell has a nucleus. The more ancient cells are called prokaryotic cells, and these cells do not possess a nucleus. Bacteria are the best examples. Most of the cells that we look at in this class, however, are eukaryotic - their cells will have a nucleus. Plants and animals are both eukaryotic organisms. Answer the questions about the two types of cells before we go on.

[24] Now we'll take a look at the tiny organelles within a cell. Record the function of each organelle in the table in your lab book as we go. Also make a note if the organelle would be found in bacteria, plant and/or animal cells. Don't worry about recording the shapes of the organelles for now. They are drawn for you later in the lab.

[25] First up is the very important cell membrane. Remember that every cell has one. I think you can remember the function of a cell membrane from just a few minutes ago, but just to help you along - cell membranes are the boundary of cells, controlling the flow of molecules that move in and out.

[26] Another organelle that forms a boundary for some cells is the cell wall. It is NOT found in animal cells, so check off only the bacteria and plant columns in your lab book. A cell wall is a tough outer cell layer that supports the cell. This will be a very easy way to tell plant cells from animal cells, just in case we ever ask...

[27] The nucleus is next. Remember, we won't find a nucleus in prokaryotic cells, so check off just the plant and animal categories. The nucleus contains the genetic material (DNA) of plant and animal cells. It really is the chemical "brains" of the cell - the nucleus directs all the activities during a cell's life.

[28] We'll take just a quick look at the mitochondrion today. It's a very important organelle that we will examine in great detail when we get to respiration. But for now, just record that it acts as the "powerhouse" of the cell, generating ATP, a chemical form of energy, for all cellular activity.

[29] Another critical organelle in terms of energy is the chloroplast, found only in plants. The function of the chloroplast is to turn sunlight into chemical energy, in other words, photosynthesis. Again, we're saving the details of this organelle for a later lab.

[30] Our next organelle is the tiny ribosome, an organelle found in every living thing, so you know it's an important one. Its function is to synthesize proteins, in other words, it assembles amino acids into the chains that compose the structurally complex proteins.

[31] The next little organelle, the lysosome, is like the stomach of a cell. The lysosome is a little container of digestive enzymes that are ready to destroy any other worn out components of the cell that are no longer needed.

[32] This organelle, the golgi complex is like a folded curtain of membranes and is the site of lipid and protein processing and packaging. When a cell needs a protein folded into a correct shape, that's the job of the golgi complex.

[33] Our next organelle is found in two different forms - rough and smooth. The "rough" endoplasmic reticulum (or "ER" for short) has a multitude of ribosomes attached to it, giving it a rough-looking appearance. Because of the ribosomes, the rough ER helps process and package the raw proteins coming out of the ribosomes. The smooth ER on the other hand, is just that - smooth looking. It does not associate with ribosomes, and its main job is to synthesize lipids.

[34] Our march through the organelles continues with the vacuole - a large fluid-filled sack easily seen in plants. It can *sometimes* be found in animal cells, but not commonly, so maybe just put a little check in the animal column, and a BIG check in the plant column. The fluid inside a vacuole is most often water, but it might also contain some pigments, stored food or toxins in a plant cell.

[35] Our last two organelles, cilia and flagella, have the same function, movement of the cell. You may have seen these organelles in action in your pond water preparation of Lab 1. If you saw protozoans zipping by, that was accomplished by cilia or flagella. Look at the two pond organisms shown here - the one on the right has many tiny cilia, the one on the left has just one large flagellum.

[36] Asking you the functions of organelles are some of a Bio 3 instructor's *favorite* questions, so let's see if you've got the idea with a couple of questions. Referring to your table, which organelles were found in all organisms?

[37] Identify the organelles that are found in plants but not in animals.

Section 4 - Bacterial Cells

[38] We'll start our discussion of cell types with the bacteria. Here you can see the variety of bacterial cell shapes. Now you might be under the impression that bacteria are all bad, but we'll learn about some bacteria at the end of today's lab that are critical for producing vitamins for you within your digestive system. You're carrying around some right now... And you undoubtedly have some other bacteria in your mouth that we'll take a look at as well.

[39] For now though, take a look at this model of a typical bacterial cell. Please record the labels on the drawing in your lab book and answer the questions about the structures as we go along.

[40] Remember that bacteria, the prokaryotic organisms, do not have a true nucleus. They certainly have DNA and genes, but this is contained in an irregularly-shaped area called the nucleoid, which means "nucleus-like". Bacteria also have some floating bits of DNA called plasmids. But plasmids and nucleoids are not the same as a true nucleus, because they are not surrounded by a membrane like the eukaryotic organisms have.

[41] Some bacteria also have a flagellum, or sometimes several flagella, as shown here.

[42] Another structure unique to the bacteria are called "pili". A single pilus is a soft little projection of the cell membrane that allows bacterial cells to stick to surfaces and to transfer cytoplasm and sometimes DNA between individual cells.

[43] Another unique structure in the bacteria is the capsule, an extra polysaccharide layer outside the cell wall of some bacteria. Now you can see that bacterial cells are pretty well-armored against the cells of our immune system. Finish Section 4 by answering the questions before we go on.

Section 5 - Animal Cells

[44] At the beginning of Section 5, you have a diagram of the typical cell organelles found in animal cells. Review the names and functions. They should all sound familiar, except the extra one shown inside the nucleus called the nucleolus. We will see these later in the semester, but for now, just remember that you may see extra bodies within the nucleus of some cells, but they aren't separate organelles.

[45] Next you will look at a living animal cell - one out of your own mouth. These cells occur in layers to protect your mouth against all the wear and tear that happens when you eat, so you always have a few extra cells that we can examine.

[46] This photograph shows how to get your sample of cells. Follow the directions carefully in Section 5 and I bet you'll be able to see some bacterial cells as well. It all depends on how long it's been since you've brushed your teeth... Continue when you have finished your drawing and measurement of the cheek cells.

[47] Did you remember to label the parts of the cell and to convert your measurement to micrometers? Great! The cells lining your mouth are called epithelial cells, and specifically a type called squamous epithelium. "Squamous" means "flat". You will be observing a different type of epithelium later in the lab.

Section 6 - Plant Cells

[48] Now we can compare your animal cell to some plant cells. We'll be looking at the cells of an aquatic plant called *Elodea*.

[49] When we looked at the thin epithelial cells of your mouth, we had to use a stain called methylene blue, but we don't need any stain to look at plant cells – they're filled with bright green chloroplasts that are very easy to see. But because we aren't using a stain, we won't be able to see the nucleus of these particular cells.

[50] Return to the program when you have completed Section 6. Keep your *Elodea* slide handy if you are going directly on to Section 7 - we can use the same leaf slide for our next exercise for diffusion and osmosis.

[51] Let's try a couple of questions before we go on. (Difference between animal and plant cells)

[52] You may want to review your lab a bit before you try this question. (outer layer of bacteria, plant and animal cell called)

Section 7 - Diffusion and Osmosis

[53] Now that we've examined the two major cell types, prokaryotic and eukaryotic, and seen some typical plant and animal cells, it's time to address how these cells function. All cells need to balance their intake of molecules, and of particular interest to us is water. Even an aquatic plant like Elodea needs to control water levels inside the cell. You'll need your Elodea slide from the previous exercise in just a minute, so keep it handy.

[54] But first, let's go over a few definitions before we discuss the process of water movement. Please record these definitions at the beginning of Section 7. A solute is a substance like salt or sugar, dissolved in a solvent. A solvent is the liquid that we dissolve the substance in, and in biology the solvent is almost always water. A solution will be the combination of solute and solvent.

[55] Now we know that molecules like solutes and solvents, can move. For example, if someone were to walk in to a room wearing a lot of perfume or cologne, eventually everyone in the room would be able to smell it. This is an example of diffusion. The perfume molecules are randomly moving across the air in the room, not because of any particular force, it's simply random molecular movement and eventually the perfume molecules will be evenly distributed across the room.

[56] So diffusion is the random movement of particles from a region of high concentration to a region of low concentration - please record that definition.

[57] Osmosis, however is a special case of diffusion involving water and membranes. Please record the definition of osmosis - the diffusion of water through a semipermeable membrane.

[58] Before we do our experiment, we need to describe our solutions based on the relative amount of solute they contain: hypertonic, isotonic and hypotonic. Notice that I said *relative* amount - in each of these situations we describe one solution in terms of the other solution it will interact with. Here are some handy translations to record on the table in your lab book - "hyper" means "more", "hypo" means "less", "iso" means "same" and "tonic" means "solute".

[59] Okay, here's a lab situation just to get us started - this bag has a 12% sugar solution inside. The bag is made of a special material that allows water to move through – it will represent our permeable membrane. If we put the bag in a beaker of pure water, we now have more solute, the sugar, inside the bag than outside. Now use your handy translations to describe the solution inside the bag - "more" "solute" is inside the bag, so it is called a "hypertonic" solution.

[60] So what about the pure water solution of the beaker? Well it obviously has "less" "solute" than the inside solution, so it would be "hypotonic" compared to the inside. Please record the proper labels on your beaker drawn in your lab book and we'll see what happens next.

[61] Now we have what we need for osmosis - a difference in solute concentration AND water on both sides. Here's what the water will do - more of it will end up on the concentrated side, in other words, the sugar side of the membrane inside the bag. Why? Because water is obeying the same diffusion principle as the perfume - it's moving to the area of its lower concentration.

[62] It's sort of like offering food at a party. If the food platters are in the kitchen, they stay in the kitchen, like the solute will stay in the bag. Every person at the party is free to move through the rooms of the house, like water molecules can move through the membrane. However, once people have found the food in the kitchen, they tend to stay there because they don't want to leave the food. Think of people as the water molecules and the food platters as the solute that can't leave the membrane.

[63] Keep this movement of water molecules in mind and complete the drawing of what happens to the bag after osmosis before beginning the *Elodea* experiment.

[64] Now you will see how a living cell reacts to a hypertonic environment. When you have finished the *Elodea* experiment, had your drawing checked by the instructor and have completed all of Section 7, come back to the program for a couple of review questions.

[65] For the Elodea experiment, what was the solute?

[66] Was the internal solute concentration of the Elodea cell hypertonic or hypotonic to the salt solution outside the cell?

[67] Now you've seen a rather dramatic reaction to how individual cells can react to their environment in relation to solutes, a nice example of homeostasis. Both diffusion and osmosis were examples of passive molecular movements - no other molecule is pushing or pulling or spending energy to move these molecules. Now it's time to look at how cells work together.

Section 8 - Tissues

[68] The cells of multicellular organisms such as plants and animals have to work together in functional units called tissues. Please record the definition of a tissue - a tissue is a group of similar cells organized into a structural and functional unit.

[69] There are four types of tissue commonly found in animals: epithelial, muscle, nervous and connective. We've already observed some epithelial cells, so now let's take a quick look at where you may find the others in a human. Please return to the program when you have completed this table in Section 8 of your lab book.

[70] You may have wondered what blood cells were doing in the "Connective Tissue" category, but blood is considered a specialized type of connective tissue. Blood cells just happen to be in a liquid matrix called plasma instead of a solid matrix we associate with other types of tissues.

[71] Next we'll examine how these various tissues are arranged within an organ. We're going to look at the small intestine of a frog because it is very similar to what you'd find in a human, but their little intestines fit nicely onto a microscope slide.

[72] Follow the directions in your lab book for observing the frog intestine slide on scanning. Find the structures shown here and complete the labels in your lab book. Note the projections called villi. A single projection is called a villus. Their function is to increase the surface area of the intestine, because the main function of the intestine is to absorb nutrients. The more surface area inside the lumen, the faster the food is absorbed.

[73] But where do the food molecules go? Although you can't see them in your frog slide, each villus has tiny blood vessels called capillaries, and they eventually deliver the nutrient-rich blood into your bloodstream. The central tube in the villus is called a lacteal and is part of your lymphatic system. The lacteal absorbs fats, so you can see each little villus is quite complicated. You can summarize the function of the capillaries and the lacteal by saying they both absorb nutrients.

[74] Now look at your slide on low power and look closely at the tall cells that line each villus. There is a darkly stained nucleus at the base of each of these cells. These tall cells are called "columnar epithelium" and are strikingly different in shape from the squamous epithelium we looked at when we observed your cheek cells. These tall, columnar cells have a much different function than your cheek cells, because it is the job of these cells to absorb food molecules as they pass by. Measure the thickness of this columnar epithelial layer before you continue.

[75] Now go to high power to examine the columnar epithelium layer in detail. You may notice the other type of cell in between the columnar cells and these are called goblet cells. Goblet cells act as tiny glands that secrete mucus, a slippery substance that keeps everything moving. I bet you didn't know a frog could be this complicated! Complete the cell labels in your lab book shown here before you continue.

[76] Now's a good time for a question.

[77] Finish up Section 8 and make sure you've finished labeling all the diagrams and answered the last couple of questions. Continue the program when you are ready to begin Section 9.

Section 9 - Organs of the Digestive System

[78] Now that we've looked at organelles, cells and tissues within just the small intestine, it's time to examine some other organs and how they work together. Please record the definitions for organs and organ systems before we go on. An organ is a group of tissues that accomplish a common function and an organ system is a collection of organs working together to perform a major body function.

[79] In the frog intestine slide that you examined a moment ago, you saw how four tissue types accomplished the common function of nutrient absorption. The intestine is just one component

of the entire digestive system, and now we will look at the other digestive organs and how they work together.

[80] Before we start, go to the demo table and examine our human model. Let's see how much you know about your body and see if you can identify the parts before I give you the answers. Fill in your guesses in your lab book. Good thing you're using pencil...

[81] We'll begin by filling in the table in your book. The first food processing that you do is obviously in the mouth. In addition to chewing, you're incorporating your first digestive enzyme from your salivary glands. The enzyme is called amylase and it starts the breakdown of starch. Record this first step in the category for "salivary glands and mouth".

[82] When you swallow, food enters the esophagus, a smooth muscular tube that connects the throat to the stomach. Other than the chewing and enzyme treatment you gave the food in the mouth, not too much has happened yet, the esophagus simply transfers the food to the stomach.

[83] In the stomach though, is where the magic begins. The food is dropped off into a pool of powerful acids and an enzyme called pepsin, which will break the food up into smaller chemical pieces that the rest of your digestive enzymes can work on. The stomach is also quite muscular, and will act to mix up the food, acids and enzymes until it is in a liquid state. Please record the processing accomplished by the stomach in your lab book before we go on.

[84] After the stomach has worked on your meal for about 3 hours, a small valve at the base of the stomach will open, allowing the food slowly into the small intestine. At this point the food is a sloshy liquid and several more digestive chemicals are added by the pancreas and gall bladder. The actual time it takes to get all the way through the small intestine varies a great deal depending on what kind of food was eaten - ranging from 15 minutes to 5 hours. Most of the time though, the food will move through the small intestine in less than 2 hours. Record the processing done by the small intestine before we go on.

[85] A normal human small intestine is about an inch in diameter and 23 feet long. That's right, 23 feet. Now you already know from looking at a frog's small intestine that an intestine is not just a big open tube. The lumen has villi to increase the internal surface area.

[86] Now put these two facts together, huge numbers of villi and 23 feet of intestine that contain them, and you can see how much surface area is contained in this organ. Some people with too much time on their hands have calculated that the human intestine has the about the same surface area as a tennis court, if you could flatten out all those little villi. THAT'S a lotta surface area.

[87] So the nutrients have been absorbed into the columnar cells, then into the capillaries and are in the bloodstream, so you're almost done with that meal. I said *almost*. The last part of our journey is the large intestine, or colon. Remember that the food had to be in a liquid state as it went through the small intestine? But when you're done with absorption, it's time to get rid of the waste, not all that water. The large intestine actively pumps salts out of the waste, causing the water to come along with it due to the change in salt concentration. Thank goodness for osmosis, or we'd all live in a *very* different society...

[88] In addition to the large intestine absorbing the extra water, it acts as a comfy home for 100's of kinds of bacteria that live in your colon. Don't panic about these bacteria - they are definitely the friendly kind because they are actually performing a service for you that you can't do on your own. They're making Vitamin K that you absorb directly into your bloodstream. So finish our digestive journey by recording the role of the large intestine in your lab book.

[89] I've mentioned a couple of other organs along the way that are accessories to the digestive process and I've listed the functions of the liver, gall bladder and pancreas for you in your lab book. Notice that they have other functions in addition to digestion.

[90] And how did you do on your guesses of the human body parts? I hope you got them all correct, especially if you're one of our future nursing students. Let's see how you did...

[91] Take a moment and finish up the last couple of questions in Section 9 if you haven't already, and continue when you are ready to begin Section 10.

Section 10 – Other Organ Systems

[92] The organs of the digestive system all worked together to get energy from the food you eat. What was a hamburger has been converted into amino acids, simple carbohydrates and fatty acids that can be transported to all the cells of your body. There's more to staying alive than just food though, so I'll mention a few of the other organ systems that are part of a human organism.

[93] In addition to the digestive system, there are several other organ systems your lecture instructor might want you to know about. I've already printed them in your lab book, so let's just take a quick tour. Here is the circulatory system, with heart, spleen, veins and arteries, responsible for pumping blood around the entire body.

[94] Here is the respiratory system, consisting of the larynx, trachea and lungs, responsible for delivering all that delicious oxygen to your tissues, working in concert with the circulatory system.

[95] Here is the excretory system, consisting of two kidneys and a bladder, responsible for fluid balance and waste removal from the blood.

[96] Here is the endocrine system, including the pituitary, thyroid and adrenal glands, responsible for regulating internal chemical levels.

[97] And lastly is the reproductive system, the testes and various seminal glands of the male, and the ovaries and uterus of the female.

Section 11 - Organisms

[98] To finish the lab today, we'll look at an entire organism, in this case an aquatic one - the common goldfish. Probably everyone has had a pet goldfish at some point, so you already know they can survive many years with just a few basics - clean water and some food. Let's think about this organism in terms of its structures and adaptations.

[99] I think you already know that fish take in oxygen through their gills, not lungs like we do. Gills are bright red because they are filled with blood vessels to pick up the oxygen from the water and deliver it directly into the fish's bloodstream. This organ has a very large surface area to gather this oxygen, as you can see from this photo of a tuna gill.

[100] One source of oxygen in the water is the air. Oxygen diffuses into a body of water from the surface of the water, so the greater the surface area, the more oxygen and the better for your fish. If you kept your little friend in a fish bowl, you probably noticed him or her coming to the surface to "gulp" air. This is a sign of low oxygen levels and your fish would really like a bigger tank or a little air bubbler to increase the oxygen supply, thank you very much.

[101] Take a moment to observe our little goldfish on the demo table if available. Notice the bright red gills and the fish's breathing behavior. When they open their mouth, they are simply moving water over the gills - they are not actually swallowing the water. Answer the questions about humans and goldfish in your lab book before we continue.

[102] And of course, it's time for a couple of questions before you go.

[103] And can you name another organ that had a large surface area?

[104] Well, that was probably more about goldfish than you needed to know, but I hope you saw the relevance of all the topics in today's lab. We looked at the interactions of molecules, membranes, organelles, tissues, organs and organ systems. I hope you liked learning about all these biological connections. That's it for this week - now go think about a bigger tank for your friend... See you next week.