>> Steve Langjahr: Tonight is February 15th, 2017. This is our fourth lecture in Bio 202. And we've discussed by now aspects of the cell membrane, certainly the importance of enzymes and what we defined as metabolism and more recently we introduced the notion of cellular energetics. How cells generate this energy currency called ATP. So as a review of what we've already mentioned, it's common knowledge that animals on this planet derive their energy source from the food, the organic molecules which we consume. But although that statement is correct, it doesn't really leave much detail because where exactly is this energy coming from? Essentially energy is released whenever bonds are broken. Whenever covalent bonds are broken, energy is released and let's be clear, we made it a big deal that energy on this planet is never created, never destroyed, just converted or transferred from one form to another. So the nature of this energy that we speak of is chemical energy and it's obtained through the dismantling of covalent bonds in organic molecules. More often than not carbohydrates, more often than not C6 H12 O6 which is glucose. Is water involved? You bet. Is oxygen involved? Yes. Are there so called byproducts or waste products? Yes. CO2 and yet more water. You can burn sugar in a test tube we spoke of that and the energy in these covalent bonds is released suddenly and completely and is dissipated as useless heat and so although this is quite exciting and impressive, it doesn't work for cells. Because cells have no way to harness heat, they can't really take advantage of heat, in fact their injured you know by too much heat. So this is something that works fine in a test tube but doesn't work fine for human cells. So there has to be a work around and we said that the work around in biological machines is to capture some of this energy and use it to create an energy transfer molecule, i.e. adenosine triphosphate. And so here's this simplistic story of what we're saying. The energy that's in these covalent bonds is actually released, not as heat, not entirely as heat, but it's transferred to make new bonds, essentially linking phosphate to existing ADP and therefore creating this high energy molecule known as ATP. To identify the players more exactly ADP stands for, adenosine diphosphate, PO4 a abbreviation for inorganic phosphate, also known, also sometimes written as PI and then of course ATP stands for adenosine triphosphate. Adenosine triphosphate is basically a nucleotide because it contains a sugar, phosphate and organic basis, but not just one phosphate it has what? Three hence the name adenosine triphosphate. Where exactly is the energy which we acquire to make ATP? Basically here in this final high energy phosphate bond. And it's the making and breaking of that which goes on constantly every nanosecond of your existence. So the energy which was available, energy which was released in the dismantling of covalent bonds, has been captured in these now brand new high energy phosphate bonds. And then that energy can be transferred, that is doled out like money to those reactions that require energy, chemical energy. So this is the basic scheme ATP is constantly being used to provide small packets of energy for biological necessities and therefore every time ATP is used, we get back a DP and the inorganic phosphate and so in short if we react, that is if we examine this reaction to the right, that releases energy, if we go to the left that requires the input of energy. We ended last time with this kind of overview of

what we know, we can expect in terms of ATP production. For every glucose molecule, that is completely dismantled, we can expect somewhere around 38 ATP. Incidentally some books will say 36, some will say 34, I'm going to stick with 38, hardly matters. But the point is that is essentially the yield. To make 38 ATP's obviously you have to have 38 ADP's and a supply of inorganic phosphate. Do we still generate the products, the so called byproducts, CO2 and water? Yes. And do we still release some heat energy? Yes. Because basically this metabolic reaction is not a 100% efficient. Because if all the energy in glucose could be transferred to the synthesis of ATP, there would be no energy left or released as what?

>> Heat.

>> Steve Langjahr: Heat. So the fact that heat is still a byproduct, testifies to the fact that this process as amazing as it is, is not a 100% efficient. In fact it's always tempting and kind of a neat, rather coincidental analogy to compare ATP to a dollar bill. And we can also compare glucose to a 100 dollar bill. So if I were to hand you or let's put it this way, if you were to give me a 100 dollar bill and I gave you 38 back would you be happy with that? No, you'd say it'd be shortchanged. But in fact that's life. Because if this is a 100 dollar bill, how much change do we actually get? 38, where did the rest go? Where did the rest of this energy go? Into lost heat. So if you're getting this math, how efficient is this process? Or if you wish, how inefficient is it? How efficient? If this is a 100 dollar bill and those are one dollar bills it's only what? 38% efficient. And that may be disappointing, but we all seem to be getting by pretty well. So those are the facts as we know them so far with respect to ATP yield. We concluded on Monday with just a statement of where this money goes, in other words how is the budget allocated? Applications of ATP. All of these are rather hungry, that is big consumers of ATP. The first is the synthesis of macromolecules. Are cells synthesizing proteins, steroids, complex carbohydrates, our bonds, our new bonds being made every second of your life? Yes and what's it take to make a new bond? It takes energy and that energy comes at the expense of ATP. So, the creation of polysaccharides from simple sugars, the synthesis of proteins from amino acids, these are big heavy duty consumers of ATP. And of course if ATP were in short supply, protein synthesis, just as an example, would not happen. And what sort of fallout would there be to that? No proteins, no enzymes, no antibodies, no membrane construction, essentially game over. So it's hard to exaggerate the importance of the synthesis of macromolecules. Then there's active transport. Active transport remember moves molecules not down a gradient it, but up a gradient it, does that require ATP? You bet. What are some of the molecules that you know are transported in this way? Amino acids are transported in, sodium transported out, these are key consumers then of ATP. Not to mention phagocytosis which is obviously an energy hungry process as well. Muscle contraction, what do I mean? Heartbeat. Muscle movement, breathing in fact, all of these are examples of muscle contraction. Do they use huge quantities of ATP? Yeah. And if you didn't have ATP obviously muscle contraction would fail and there you

are, you're dead because you're not breathing and you're not moving blood. So once again muscle contraction, huge consumer of ATP. And last cell division, which is an impressive process to witness in videos abound on this. Essentially one cell becomes two and two become four. An incredible process, needless to say consuming ATP. So whether we're talking about mitosis or meiosis, big user of ATP and without it obviously cell division would stop and you would be on a slippery slope to, well to the grave. So these are intuitively obvious uses for ATP and therefore testified to the importance of that molecule. So now let's move from that into more specific dialogue that is we're trying to now explain more in detail where ATP comes from in human cells. We gave you a kind of sweeping overview where we stated that 38 ATP molecules are formed. But we were careful to point out that it's hardly one reaction. In fact you might have noticed the fine print on the previous page. It said that hardly one reaction there are 19 steps, 19 reactions in that big picture simplification. And that is of course the definition of what we call a pathway. A pathway is a sequence of many enzyme mediated reactions that produce intermediate products ultimately a terminal product or two. Intermediate means just that, terminal means at the end. So just as a working example this could be a representation of a metabolic pathway. Substrate a is converted to product b and that product becomes a substrate which reacts with g to form c and then onward into e or f, notice that enzymes are involved in every one of these reactions. So this is a simplistic, generic representation of a metabolic pathway. What are the intermediate products? What are the terminal products in this case? It's tempting to say that g is a terminal product, because it comes after f. But actually g as you can see reacts with b to form c. So all of these are intermediate products except the only one that apparently does not change and that seems to be d, so by definition a terminal product is an end product, an end product that doesn't get converted or used in any other way and very often eliminated from the cell as surplus or as a metabolic waste product. So all of these intermediates with only d being the terminal product in this case. Now metabolic pathways come in two very distinct forms. Essentially those that release energy and those that consume energy. Remember we said metabolism as a word, as a concept, includes all the reactions that are happening in a cell. But some of these reactions use energy, some of them actually release energy. So let's divide and define the two sub-kinds of pathways. First a catabolic pathway. Sometimes also called simply catabolism. Catabolic pathways break chemical bonds and therefore make available or release energy, thus at least providing the opportunity to make this energy currency ATP. The most important catabolic reactions in the human body are those that are part of what's called cell respiration. Cell respiration is the generation of ATP, typically at the expense of carbohydrates. The opposite of a catabolic reaction is a anabolic reaction. You might have even heard that term, maybe in a negative connotation, perhaps you've heard the term anabolic steroid, anabolic steroid. Anabolic simply means to build up and so these are pathways that actually synthesize molecules, therefore creating new bonds and more complex molecules. That said, if you're making bonds what are you going to need? What do you need to make chemical bonds? You need

ATP. So clearly anabolic pathways, important as they are, are big consumers of ATP. And the most important of the anabolic pathways are those that lead to protein synthesis. What's so important about proteins? Do we have to say it again? Are enzymes proteins? Antibodies proteins? Cell membranes proteins? Muscle contraction proteins? It's impossible to exaggerate the importance of protein and so the most important of the anabolic pathways are those that create or synthesize proteins. Now these two subtypes of metabolism, catabolism and anabolism are sort of intransigently linked, that is one depends on another. And so we say the catabolic rates tend to match or equal what? Anabolic rates. That might be easy to memorize, but it's easier to understand in the context of your own home economics. Catabolic generate money don't they? Anabolic what? Use money. Is the amount of money you make going to limit the amount of money you spend you think? Yes. So that's the essence of that concept. Anabolism can't run any faster than catabolism, because you can't spend money you don't have. And so catabolic rates tend to match or equal anabolic rates. And we can say further that catabolic reactions are mutually dependent on anabolic reactions, what's that mean mutually dependent? One depends on the other, that is they are linked, they're inseparable. Let's make that clear with a diagram and let's restate catabolic pathways break down molecules and produce what as an important product? ATP. Anabolic pathways consume this ATP and generate what in return? Mainly molecules, complex molecules such as protein. This makes it fairly obvious that anabolic pathways are dependent on catabolic pathways, but how are catabolic pathways dependent on anabolic pathways? What protein drives or makes possible catabolic reactions? It's the e word up there what? Enzymes. So it's one thing to say protein, but we're really talking about enzymes. So in case I'm not clear, what do catabolic pathways provide that make possible anabolic pathways? ATP. What do anabolic pathways provide which drive or make possible catabolic pathways? Enzymes. That's what we mean when we say they are, what mutually dependent, you can't have one without the other. Not only are they mutually dependent, but the rate of one tends to influence the rate of the other. Taken together catabolic pathways and anabolic pathways are basically metabolism. Metabolism then essentially catabolic working with anabolic pathways. And the time we have left and as we move even to next week, we're going to first talk about catabolic pathways, then at the end of this particular unit naturally we'll talk about protein synthesis, which is the biggest example of anabolic pathways. So as an introduction to the lab on Tuesday and an introduction to this topic, cell respiration. Cell respiration essentially include catabolic reactions which provide the cell with? ATP. And we've introduced the simplistic expression of this notion. What is the typical, the most available, the most sacrificial primary substrate whereby we obtain ATP? It's right here, glucose. You might say well I don't eat glucose, so it doesn't apply to me. Well you might not eat glucose but all of you eat carbohydrates and carbohydrates that you eat are converted to? Glucose. And then there's glycogen which is stored in your liver and you know glycogen is just basically a bunch of glucose molecules. So you might think it oversimplified but most of the energy in our body comes from the breakdown, the catabolism of

glucose. As we said already is water involved? You bet. Is oxygen preferred? You bet. Are there products which are essentially terminal and also at the same time useless? Yes, CO2 and water and also heat. We list these as waste products, what happens to the CO2 generated as a result of cell respiration? We exhale it. What happens to the water which is produced as a result? That's exhaled too. You might not think you exhale water, but just exhale on a piece of glass and what do you see? Water. So water and CO2 are both eliminated through the lungs. Heat is also eliminated through the lungs, but heat is also eliminated from every square inch of our body so, these are indeed surplus and are eliminated by one mechanism or another. What's important then are not the waste products, but the payoff, where's the payday? ATP. And we've already given you a number, which is somewhat in dispute, but to repeat for every glucose molecule, what's the maximum yield we could hope for in terms of ATP? 38. Just a estimate of that yield. So we're now going to talk about exactly how phosphorylation occurs. The word might seem strange at first but it really is what it sounds like. To phosphorylate is to add a phosphate group to an available or existing ATP. So in short this is phosphorylation, adding a phosphate to an existing ADP. And therefore making ATP. There are only two methods of making or phosphorylating ADP in human cells. And so we'll talk about these with the period that we have left and the first of the two, substrate phosphorylation. We'll start by saying that substrate phosphorylation is very simple, very straightforward, but also rather rare, in other words this process occurs very rarely. It essentially is a single direct attachment of a, I should say phosphate onto available ADP. So this is the simplistic expression of what we're trying to say. Here's a substrate, we're leaving it unnamed. Obviously it's a organic molecule, you see what there? Carbon. So we're not going to identify by name, let's just call it a generic substrate, what's interesting about it is that not only is it organic, it apparently has what attached? A phosphate. And what apparently happens, at least what seems to be suggested here, is that this phosphate is taken off that substrate and added directly to an available a, to make available ATP and clearly the product now is one phosphate deficient. This requires an enzyme, not surprisingly it has a name that reflects what's happened here, it's called phosphatase and that's going to be the summary of this process. Now this process as we've said is a single step reaction and all it really amounts to is removing a phosphate from a substrate and transferring it onto an available ADP. From what we're said and for what we show is oxygen involved in this process? No. Is any special organelle mentioned or required? No. So this kind of event does not depend upon mitochondria, it certainly doesn't include or require oxygen and so generally it's occurring throughout the cytosol, throughout the cytoplasm, without the involvement of any fancy organelle. Is oxygen needed? No. Does oxygen interfere with this? No. So essentially oxygen is a, well a nothing in this and so substrate phosphorylation, straightforward, easy to understand. The disappointing thing is what we've already said, is this common or uncommon? Very uncommon. In fact as we look to the budget, as we look to the money production in the cell, only 10% of the income is generated by this means. Meaning what? 90% comes from somewhere else. So with

that said could you survive on substrate phosphorylation you think? If I said to you whatever your income is we're going to take away 90%. You going to be okay with that? Probably not. So cells obviously would have a hard time, however, is 10% better than no percent? Are there times when substrate phosphorylation can really be handy? You bet. It's handy because as we've said it doesn't require what? Oxygen. So are there conditions in cells or tissues or organs where there might be a shortage of oxygen if only briefly? And therefore what would have to be resorted to would be substrate phosphorylation. Would that get you by for the rest of the day? No, but would be good in a pinch? Yes. So I don't want to label this as a backup plan, but it does provide at least some ATP, especially when oxygen is in short supply. So where's the 90% come from? Clearly oxygen is involved as we know we'd all be dead without it. So the name of this kind of phosphorylation is called, oxidative phosphorylation. It's an indirect and multi-step process far more complex and obviously involves oxygen and as we'll see shortly it requires the involvement of organelles that you know to be important so called mitochondria. So as we unveil this we said it's not a single step but a multi-step process, let's begin at the beginning. Here's an initial reaction that we'll lead to and be part of oxidative phosphorylation. We see a substrate or at least part of a substrate illustrated and notice incidentally that there's no phosphate group on there. But we do see and appreciate that it's an organic molecule with covalent bonds and as you look to the product, what is happened, that is how would you describe the difference between the product and the substrate? Apparently hydrogen's have been lost, right? And so the name of this phase, this initiating step is a dehydrogenation reaction. Which is intuitively obvious, are we removing hydrogen's? How many at a time it seems? Two. And therefore not surprisingly the name of the enzyme that makes this possible is called a dehydrogenase. There are very specific dehydrogenases for each of the many substrates that might be involved in any given dehydrogenation reaction. But again we're keeping this generic just for simplicity. Now I know you're unimpressed at this point, you're saying okay we've lost two hydrogen's, but wait a minute where did they go? They weren't just cutoff into space, apparently those hydrogen's which were on the substrate have been transferred to a molecule involved and in fact working with the dehydrogenase enzyme and the name of this molecule is NAD. I know it's tempting to call it NAD but we're not going to get that chummy. We're just going to call it what? NAD. Which is an acronym for nicotinamide adenine dinucleotide. Now you don't have to memorize that, but the important thing about it is that NAD is a coenzyme. A coenzyme as you know, coenzymes are derived from? That is synthesized from water soluble vitamins, in this case NAD is made from a vitamin you know by name, niacin. Okay. So what's the coenzyme doing here? Let's be clear, is NAD a protein? No. Is it an enzyme? No, it's a coenzyme. Does it take the place of dehydrogenase? No. Does it work with dehydrogenase? Yes and exactly what does it do? Well apparently it picks up hydrogen's, how many? Two. From what? Here are two hydrogen's, you seem them there? Where were these previously? Those were right here and right here, now they're over here. So the function of this and other coenzymes is to receive, to accept, to transfer

hydrogen's off of substrates. To repeat the enzyme that makes this possible is not NAD it's? Dehydrogenase. So this mechanism is the first step in what we're describing here, namely oxidative phosphorylation. The name of the coenezyme was? NAD. Later we're going to discover that there's one more player which is rarely used but essentially does the same thing, it's name is FAD, which stands flavin adenine dinucleotide. But both of these show the same basic capacity, they remove what from what? Hydrogen's off of substrates, with the involvement and participation of? Dehydrogenase. Now, okay fine, but do you see any oxygen mentioned or involved at this point? Do you see any ATP mentioned involved or even produced? No. So clearly we haven't done anything that is energy related yet, all we've done is pick off what from what? Hydrogen's and the name of this reaction is a dehydrogenation reaction. Occurs many many times in the course of cell respiration and we'll go to that in detail next week. So I know you're begging to know what happens next. Where do these hydrogen's go and who cares? Well basically these hydrogen's are going to be escorted, transferred to an organelle which is available often times in great numbers and the name of that organelle we've mentioned already, the mitochondria. So if you'd like NAD and FAD are escorts, they basically carry hydrogen to the mitochondria, passing through the mitochondrial membrane and dumping these off inside the mitochondria. Before we go further we need to of course say a bit about hydrogen. Hydrogen is the simplest atom known and if you recall some chemistry, a hydrogen contains how many protons? One. How many electrons? One. How much more simple can that be? So if we talk about a hydrogen atom it's made up of one p word, proton and one e word, electron. If we then dismantle this atom, we're going to get a proton and one electron. There are many ways to express a proton, we can use the positive side and in fact when we go h plus you can call that a hydrogen ion or you can simply call it a proton. To repeat every hydrogen is made of one proton and one electron. So let's continue our story. We know that this process is initiated at random in the cytoplasm, but it's going to be concluded, that is the final steps and the important payoff is going to occur in the mitochondria. And incidentally obviously if a cell has no mitochondria what can it not do? If a cell has no mitochondria I cannot carry out what? Oxidative phosphorylation and that's a bit paradoxical, I'll digress for a minute. Because what cell do you know of has no mitochondria? Red blood cell. And why is that ironic? What does a red blood cell carry? Oxygen. What is that ironic? It's carrying all this oxygen but guess what? It can't use it. Why not? Has no mitochondria. And before you start you know weeping over that it's actually a good thing. Because if a red blood cell had mitochondria it would use the oxygen and therefore its function which is to deliver oxygen would be undercut. It would be like RBC saying well I was suppose to deliver this oxygen but I got sort of greedy and I used it on the way. So I know that's completely off topic, but it is unexpected, the cell like a red blood cell, although it's loaded with oxygen what? Can't use it because it doesn't have what? Mitochondria. What is the role of oxygen as we're about to unveil? We tend to have this exalted notion that oxygen feeds the fire of life and it makes life as we know it possible, I suppose that's all true. But the rather deflating

reality is that oxygen is the final electron acceptor in a sequence we're about to describe called the electron transport chain. And this chain, the ETC what? ETC, electron transport chain was originally called and still is in some older books, it was originally named the cytochrome system. And here's a drawing then, a cutaway, a slice through, a typical mitochondrian. We've already that it's a membrane bound organelle, actually it's two membranes, the outer membrane rather smooth, the inner membrane is folded or pleated into these shells called cristae. So this may or may not be familiar but this is the micro anatomy of this organelle. Some cells have no mitochondria, some have hundreds even thousands just as a random question. Compare a liver cell to a sperm cell? Which you think has more mitochondria? Liver cell. 800 at least, sperm cell would be lucky to have 20. What does that even matter? Clearly the number of mitochondria that a cell has dictates the level of oxidative phosphorylation that it's capable of doing right? So we find a correlation, those organs that are busier, more active, more important are naturally expected and likely to have more what? Mitochondria. A direct correlation which we'll elaborate on actually in Tuesday's lab. The working parts of the mitochondria are in fact molecules called cytochromes. They were called cytochromes, because chrome, the word chrome means color. And under the microscope these molecules were bright red. Turns out they're red for two reasons, they are iron containing proteins and there's also copper in these cytochromes too. The cytochromes then are small iron containing insoluble proteins that are arranged side-by-side, shoulder-to-shoulder along these inner folds of membrane forming essentially independent structures in the cristae. Cytochromes, hence the name of the system what? Cytochrome system. And is okay as that is, it doesn't really tell us anything about it, if we say cytochrome system is there any functional reference in that? No, cytochrome, colored cell and it's kind of a meaningless term, so today more often than not it's called the electron transport system or the electron transport chain because even though we haven't yet discussed it, is there some functional reference there? Do we have some idea what's going on? Apparently it's a chain, which transports, electrons, at least we have something to go on here. So now let's move into and actually dissect the essence of the ETC, what's ETC? Electron transport chain. Quite sure there are videos on YouTube about this. But I hesitate to you know launch you into that jungle because some of them are dumbed down to the point that they're useless, some are so sophisticated it'll blow your mind. So we'll try to give you sort of the, you know middle of the road explanation of what's going on. Enough so that you appreciate the components and appreciate the value and importance of the ETC. So we're going to simplify the mitochondria, we're going to diagram it as a box within a box. So this outer line here is going to be the? Outer membrane, the inner line here is the? Inner membrane, we're simplifying it, we're making it diagrammatically easier to follow. So returning to where we left off and just recapitulating, substrates are stripped of hydrogen's in the cytoplasm. How many at a time? Two. What are the names of those reactions where hydrogen's are cleaved off of the substrates? Dehydrogenation reactions. Are there enzymes involved? The name is? Coenzymes involved? Most common player here is NAD but an occasional

sort of substitute FAD. So we left you with NAD. NAD has acquired how many hydrogen's? From substrates. Again with the involvement of dehydrogenation or dehydrogenase reactions. So here we show it, this is where we left you out, this is where we start our board game if you want to think of it in that way. These hydrogen's are escorted into the interior of the mitochondria and are essentially let go. Obviously releasing and returning what back to the cytoplasm? NAD. So there's an important observation. Is NAD consumed, depleted, destroyed or injured in this process? No. Obviously it's recycled. That is NAD picks up, releases, picks up, releases, constantly essentially moving between and from the interior of the mitochondria out to in some cases the cytoplasm and so on. So we've dumped off hydrogen's and these hydrogen atoms are going to be then separated in to two e and two h plus. 2e what's that? How do you say it? Two electrons. 2h plus? You can say two hydrogen ions, but you can also say two protons. So you take your pick. 2e, two electrons, 2h plus, two protons or two hydrogen ions, this is not an atom, this is a hydrogen ion, which has a positive charge. Now remember the name of the system, which was first called the cytochrome system is actually more commonly now known as the? The electron transport chain. So clearly our focus is on the electrons, which are going to be transported actually, that is exchanged, passed along a series of proteins we've already identified as cytochromes. To repeat cytochromes are small, iron containing, insoluble proteins arranged side-by-side, shoulder-to-shoulder so to speak along the inner membrane of any given mitochondria. These cytochromes have the capacity to accept one electron and then pass it to the cytochrome next to it. And so this is the essence of the electron transport chain, we show how many electrons waiting or otherwise dumped at the doorstep here? Two, but to repeat a given cytochrome can only accept one electron and then it will pass it to the cytochrome next to it and so on and so on. The exchange, the passage of electrons is the very definition of electron transport chain and each of these exchanges releases a small bit of energy. I know this is far fetched and it's not real at all but it reminds me of a game that use to play as a kid it was called hot potato and you would get a hot potato out of the oven and you would pass it, what from? Person to person. Now why is that relevant? Well as that potato goes down from person to person it losses what? Losses heat. So in a similar way the passage of this electron or these electrons releases energy but as we move further along this chain the energy available becomes less and less and less. Now what happens to this energy as it's released in the exchange or transport from one cytochrome to another? That energy is used to pump, p u m p protons which are plentiful here in the interior into the space between the inner membrane and the outer membrane. In fact these are protons yes? So this is moving those protons against a gradient, from a what? From a low concentration into an ever creasing high concentration. So this is called a proton p word? Pump, a protein pump. And the energy to do this is obtained as a result of the energy released in the transfer of these electrons. We'll animate this for you later but for now you got to use your imagination. So to repeat an electron is going from cytochrome to cytochrome, energy is released and that energy is used to move what from where to where? We're going to move protons from here into here.

Down or up a gradient? Up a gradient and therefore naturally depending on the energy released in these electron transfers. So what you say, now we've got a huge concentration of what where? A huge concentration of protons in this space between the inner membrane and the outer membrane. Do we have an extreme concentration gradient? Yes. Which way would protons tend to move if given a chance? They would tend to move from a high concentration here to a lower concentration in the interior matrix of the mitochondria. And they have, three such opportunities along this sequence which are shown here as channels which allow the return of protons back into the interior. The movement, the physical movement of protons through these channels releases energy enough to tack what onto what? The energy of the movement of these protons is enough to attach a phosphate to an existing what? ATP, thus producing apparently one ATP, all right whoopee at least we've got something to show for this. But we're not done, we're only at the beginning of this electron transport chain. The next sequence is another cluster of cytochromes and the same electron is going to be transferred again from cytochrome to cytochrome. To repeat how many electrons can a cytochrome accept before it transfers? One, so got to lecture on, okay get rid of it, get ready to get the next one, essentially like a bucket brigade, I don't know if that makes any sense but in ancient times you know a fire was there, you'd pass the bucket from person to person. The electron transport chain releases energy enough to do what? Enough to pump what from where to where? Protons from this area into the space between the membranes, is that down or up a gradient? Up. So we again build this concentration of protons in this space between the inner and outer membrane. Will those protons sneak back in? Yes. They'll go through channels, this second of three channels and the movement, the diffusion of these protons back into the interior releases energy enough, enough energy to add a what to a what? A phosphate to an existing and available ADP, thus producing what? So far two ATP's. Now wait a minute, we notice something else that we seem to have overlooked. We mentioned previously NAD as the key player, the key coenzyme, but we also at least named FAD, flavin adenine dinucleotide. Notice that it too picks up hydrogen's, but a strange difference it has a lower energy value and it cannot start this game from the beginning, it comes in midway. And therefore its protons, its hydrogen are going to start here rather than at the beginning of this story. We'll have more to say about that, but felt compelled to at least mention it, we're not done. So these electrons, again one at a time, have a final, final row of cytochromes to traverse. And once again each cytochrome can only take, can only accept, can only pass one electron at a time. This releases energy, energy enough to drive what or pump what from where to where? Moving protons from here into here, that's called a proton pump which elevates the concentration here and therefore creates a gradient which would favor the return of protons, that is the downward diffusion of protons through this final, this final channel which releases energy enough to do what? Enough energy to tack on a phosphate to an existing ATP, thus producing? Three or our third ATP. Great. Now this process of releasing energy and phosphorylating the ATP is also dependent on an enzyme, which is marked here by an asterisk, an enzyme which is part of and present at these

channels, the name of that enzyme is ATP synthase. And so obviously without that enzyme there would be no ATP made, even as energy was released then. Are there any more cytochromes as far as you can tell? Nope. So we seem to have reached the end of the ETC, electron transport chain. Which of course raises a question, what happens to these electrons now? A extremely important question. Because if this final cytochrome could not get rid of the electron, that it just acquired, it could not accept any others yes? And therefore this whole process would stop and back up and everything we mentioned would s t o p right? So something has to happen to these final electrons. The buck has to go somewhere. And here it is, the rather disappointing but glorious involvement of what we know we inhale and depend on what? Oxygen. We said earlier that oxygen is the final, the ultimate electron acceptor. I don't want to say it's waiting at the end of the electron transport chain because oxygen is everywhere, but oxygen is hopefully there. And let's look at it. Oxygen exists in the atmosphere as O2, but all we need here is half of that, in other words half of O2 and think about it, if we have an oxygen atom and if we have some protons and if we have electrons. Think about it, half an oxygen, two protons, two electrons what does that make? Water. So the end product of the electron transport chain is not only ATP, but we're also making what? Water. Now this might be kind of stunning to you, because we know we consume water, we know that we depend on water. But the interesting fact is the human body actually makes water, makes water from what? Oxygen and the hydrogen's. Where did these hydrogen's come from, let's not lose sight? They came from the? Substrate. So does the human body make water? Yeah. Do we care? Not really, because usually there's plenty of water. So it's not the water that we care about, but you might still ask where's this water go? Well I don't know, it just goes. I mean obviously you can't say where did you come from water. You know might have come from [inaudible] or otherwise out of here. So water is water and is there in the human body usually a surplus of water, usually a surplus of water? Yes. So really don't care where that goes, the important thing is that it's an innocent innocuous molecule, does it do any harm? No. And the importance of that reaction is not making the water, but essentially getting rid of or finding a home for what the? Electrons and the protons for that matter. So what if that reaction were to halt and indeed it would halt without what? Are there times in the human body where oxygen is in short supply? Will this system shut down in the absence of oxygen? What do call that, there's a name for that, there are many names for that, one of them is death. But what do you call it when there's a shortage of oxygen? You can call a asphyxiation, more commonly it's called hypoxia, ever heard of hypoxia? How do you become hypoxic? There are many ways. You can become hypoxic by climbing Mount Everest, you can become hypoxic by having someone strangle you and you can become hypoxic if you have low RBC's, why is that? Low RBC's, anemia, you don't have those cells you're not going to transport? Oxygen. There are many ways to become hypoxic, but the bottom line is if you are hypoxic this process, what's it called the electron transport chain is going to slow and maybe even stop and if it stops, game over, why? What percentage of ATP that's made in the body is made as

a result of the ETC? 90%, can you live on ten? No. So pretty catastrophic. Anything that interferes then with oxygen transport or oxygen availability is going to cripple this process and spell death for the individual. Even more interesting and somewhat shocking and sad, is there are things in nature which block this final reaction even in the presence of oxygen. The most notorious and familiar molecule that does that is cyanide. What's the reputation of cyanide? It's a poison, it's a deadly poison and it's a simple explanation because cyanide blocks this final reaction preventing electrons from moving from the final cytochrome to what? Even, no to water to, oxygen to make water right? What if you prevent this electron from joining up with oxygen? Well then everything backs up right? Everything backs up and the entire ETC shuts down. What happens to your ATP yield? 90% gone right? Are you dead right there? You are. Hydrogen cyanide, very deadly and well here's a name, Jim Jones. Nobody seemed to react, look this up, Google it. Until 911 the single one day loss of life in a non or in a peaceful environment, the single one day loss of life, the record until 911 was in 1978 in Guyana. Jim Jones the Peoples Temple was, I don't what he is, he was a guru, a demagog, a crazy man, and he had followers down there and convinced them to drink the Kool-Aid, in fact isn't that practically a catch phrase these days. I drank the Kool-Aid, what does that mean? He put cyanide in Kool-Aid and had his people line up and 909 of them lined up and were dead in an hour, right there. Look at this, it's just catastrophic, 300 of them were children. Did Jim Jones die that day? Yes, but he didn't drink the cyanide, he shot himself in the head, he took the easy way out, it's a horrible story. Why am I bringing it up? Because it testifies to the what, the toxicity, the toxicity of cyanide. And actually it's not that toxic, what do I mean by that? It only does one thing, it blocks what the? Electron from moving into oxygen, is that good enough to kill somebody right now? Yeah. So cyanide is a pretty awful compound for that one simple reason. So let's go back up and repeat because we, well said a lot. These hydrogen, hydrogen atoms are taken off what with the assistance of? Off of substrates with the assistance of an enzyme called dehydrogenase, coenzyme involved usually is? NAD, escorting these into the mitochondria. These two hydrogen atoms are separated to into and e and p, that is electrons and? Protons. The electrons are exchanged from cytochrome to cytochrome. Energy is released in this transfer and pumps protons from here into there generating a high concentration of? Protons. Which then sneak back, sneak back that is move back down a concentration gradient, releasing that energy and making, that is allowing for the synthesis of ATP by tacking a phosphate onto ADP. Enzyme required and available at each of these gateways is ATP synthase, how many does this happen? If the protons are delivered on the back of NAD one, two, three. Now what if the messenger was not NAD but instead FAD? FAD cannot and does not start at the beginning of this sequence, but rather sort of midway. So what's the difference if we start with NAD we can expect one, two, three? But if we start with FAD we're only going to get one, two, that's just a fact, don't ask me to explain it because it's just a fact. But it does have an impact in the overall tally. So with that said let's look at the overall math involved, that is the oxidative processes as we've described it. Here's

our coenzyme it's NAD and it delivers two hydrogen's and as long as there are three ADP available we can expect what? We can expect, here it is, three ATP. What are the byproducts or even end products of this? Is NAD returned for circulation? Is NAD recycled, returned to duty? Yes. And then we also get H2O. Do we care about H2O? Yes and no, I mean it certainly important but it's just water, our real focus after all is in the money, where's the money? Right there. Now FAD is a little different, it comes in not at the beginning but sort of midway in the ETC, therefore only two opportunities to make ATP and that then is the total with FAD. Now we gave you a number, a number for the approximate maximum ATP production from a single molecule glucose, what was that number? 38. Do they all come from this? No because remember we said this at best is 90% what? 10% come from? Substrate phosphorylation. But in terms of the importance of this it's impossible to exaggerate it because without mitochondria and without the ETC, ATP as we know it would fail and therefore what would suffer were the four biological processes which depend on and would grind to a halt without ATP. Flip back a page, synthesis of macromolecules, active transport, muscle contraction, mitosis. So speaking of cyanide how does cyanide kill people? Well we told you it's knocks out the ATP but ultimately it's cardiac arrest and? Respiratory arrest, both of those are examples of muscle contraction which will stop right now and of course normally when your heart stops that's pretty much it. So as sad as it is cyanide testifies to the importance of that. Also and I guess I'm kind of ranting at this point, you've heard of carbon monoxide. Carbon monoxide we think of as a poison, it's not really but what it does is it blocks the attachment of oxygen onto the hemoglobin molecule all right. Blocks what? The attachment of oxygen onto the hemoglobin so what are cells not going to receive in the presence of carbon monoxide? Oxygen. So once again that's the h word, that's hypoxia and so does carbon monoxide cause death? Yes through what? Hypoxia. But the bottom line is not hypoxia it's a lack of ATP and therefore the lack of muscle contraction and everything else. So you know we can speak of oxygen, we can speak of red blood cells, but it's really a matter of money and it's ATP in the end. Now without dragging this on, we did say that maybe 38 ATP's are made, we mentioned CO2 although we haven't shown it here, we'll get to that on Wednesday. But is there still heat produced in this process of glucose catabolism? Yes. Because we've said this process was not 100% efficient. And where's that heat actually released? The heat is released in the transfer of electrons from the cytochromes. So although we don't mention heat, is heat being given off from these mitochondria? Yes. And let's ask the question, is heat a good thing or a bad thing? Both because a certain amount of heat is necessary to provide the important activation energy. But does the body usually have plenty for that? And so overall is the body trying to conserve heat or get rid of heat most of the time? Get rid of it. If we could take a thermal graphic photo of everybody in this room, we'd all be aglow with red and there would be heat rising up, in fact is this room getting warmer just because we're here? You bet. So heat is normally a surplus commodity and it comes because of the inefficiency of this process. Now like it or not luckily we are able to get rid of that heat and so it's not normally a problem. In fact

there is some benefit to that heat and that is to provide activation energy. So how about a question? How about two? How about anything? It's a lot isn't it. Important for you as I've always said, is not just to listen to the podcast and say yeah I understand that, yeah I understand that, you have to then turn it off and speak to your dog. You have to explain it to your dog, you say well he doesn't really care to much for this stuff. But I'm just saying talk to the wall because if you can't explain it then you don't understand it. So that's how you do it and if you have trouble text me or otherwise get on the phone. Good.

>> So I know you said that 10%, we have, we obtain 10% ATP from substrate alternative in people and then the other 90% comes from electron transport chain? But is it pretty much from oxidative phosphorylation because?

>> Steve Langjahr: Yes. That's a great question. What's the difference between the electron transport chain and oxidative phosphorylation? Well they're the same thing, the electron transport chain is the concluding, the concluding part of oxidative phosphorylation. In other words if I point to this ATP, how was that made? Was that made by substrate phosphorylation or oxidative phosphorylation? Oxidative, oxidative, oxidative because without oxygen would any of this happen? No. So the electron transport chain is the actual concluding aspect of and essentially optimizes oxidative phosphorylation. Now I'm going to shut this off I can show you a little animation I made, it's only five minutes so hopefully that will help and we can even walk through it together. But let me at least.