[SQUEAKING] [RUSTLING] [CLICKING]

PROFESSOR: OK, good morning. So today, we're going to begin our discussion of nitrogen metabolism, which is really going to continue over the final three lectures in the class.

Now, nitrogen, of course, is critical for life. You need nitrogen to make RNA and DNA, as well as protein. And so what we will see is that as we discuss the metabolism of amino acids, and nucleic acids, not so much how they contribute to protein and RNA and DNA but rather how they interface with other pathways and metabolism to lead to how these molecules are synthesized, how they're broken down-- and what you will see as we go through these discussions is that understanding the metabolism of these molecules really is all about how organisms handle nitrogen.

Now, of course, we've spent much of the class talking about how fat and sugar can be oxidized as a way for cells to get energy. And of course, that's very important. A major role of fat and sugar is energy transduction.

But of course, cells are mostly made out of protein. And so roughly 50% of a cell by mass is protein. And protein, therefore, is a key part of our diets, and really, any animal's diet that eats other organisms. So anything that's nonphotosynthetic has to be taking in a lot of protein because it's eating other organisms in one way or another.

Now, you will, of course, know that nucleotides are central for and the heritability of life, and so life couldn't exist, at least in a heritable form, without those. And often, we equate things like amino acids, and nucleic-acid metabolism, nitrogen metabolism as all about energy and weight-lifting, so weightlifters-- all the supplements that are sold to help people get huge-- all of those things usually involve heavy loads of nitrogen-- so creatine-- we talked about that earlier in the course. Leucine is a very common weightlifting supplement.

These are all nitrogen-containing compounds, and it makes sense because if we're going to build new mass for cells, we certainly need nitrogen to build the protein-- that's what half the cells are-- as well as the nucleic acids that enable cells to go on and build new cells. And in fact, roughly 50%, as I said, of the mass of a cell is protein, and another 10% is nucleic acid. So really, a large chunk of what our cells are comes from these nitrogen-containing compounds.

However may be something you don't think about as much is that we also, because we have to eat other organisms, consume a lot of protein in our diet. And therefore, protein and particularly, different amino acids, also end up being a very important nutrient. And in fact, our muscles and our liver are storage organs, if you will, for amino acids. And so our muscles are always getting bigger and smaller, breaking down, rebuilding each other as we go through life as they really participate in our body's metabolism.

In fact, you have tons of energy built up into your muscles. And if you starve yourself, stop eating, you can live off of muscle tissue for quite a lot of time. And that is really using the amino-acid breakdown from our muscle tissue, really, there as a way to keep glucose in our blood high and keep our brain thinking right. That's why we talked last time about the keto diet.

When we talked about the keto diet, remember, if you're going to do this properly and really be ketogenic, you can't eat glucose, of course, but you also can't eat-- or carbohydrates. But you also can't eat protein, and the reason is because the liver will turn your amino acids back into glucose. And that's true whether those amino acids come from the diet or those amino acids can come from the breakdown of muscle. All right.

So if we use amino acids as a source of energy as a way to support gluconeogenesis to keep our carbohydrates available for our brain, we need a way to metabolize those amino acids. And if we metabolize those amino acids, we also then need a way to deal with all the excess nitrogen. And so, indeed, we already introduced how this works.

And if you remember back when we talked about the malate-aspartate shuttle, which you'll recall was a way for us to transfer electrons from the cytosol into the mitochondria and involved this convoluted swaps between aspartate, and oxalacetate, and glutamate, and alpha-ketoglutarate, and at the time, we introduced how amino acids are related to alpha-keto acids. And so I draw out how oxalacetate, an amino acid, is related to the alpha-keto acid-- sorry-- how the amino acid aspartame is related to the alpha-keto acid oxalacetate, as well as how the amino acid glutamate is related to the amino acid alpha-ketoglutarate.

Just to be very explicit remind you what I meant for this, and so here is the amino acid alanine. This is the amino acid alanine. It is related to the alpha-keto acid pyruvate.

And again the same relationship, if you look back in your notes when we discussed the malate-aspartate shuttle-- glutamate to alpha-ketoglutarate, aspartate to oxalacetate. Really, the keto group, the alpha-keto group, is being replaced with the amino group. And what should be clear to you now is that if you can convert an amino acid into an alpha-keto acid-- that is dispose of the nitrogen, get the ketone back-- it should be very clear how you can then oxidize pyruvate, oxidize oxalacetate, oxidize alpha-ketoglutarate-- as a way to get energy directly, burn it in the TCA cycle, get ATP, NADH that way, or turn it into glucose by gluconeogenesis.

However, if we're going to do this, obviously, these nitrogenous have to go somewhere. And if you're going to build amino acids, those nitrogenous have to come from somewhere. And that's really why the pathways that we're going to discuss really, are about nitrogen metabolism and how cells deal with either the excess nitrogen from breaking down amino acids and nucleic acids or how cells obtain that nitrogen as a way to build these molecules.

Now, by way of introduction, we have to discuss also, where is it then that nitrogen, for biological systems, really comes from in the first place? Now this is almost never an issue for animals, and that's because we and other animals eat other organisms. And so we eat other organisms-- usually, we have a problem of dealing with too much nitrogen.

However, for photosynthetic organisms, how to get nitrogen ends up being a real problem. And so any of you who have done any gardening know that you need to add fertilizer to your plants. What is fertilizer? Well, it's primarily a source of nitrogen, as well as phosphorus. And really, it's the nitrogen in the fertilizer that's helping solve the problem of where the photosynthetic organisms are going to get the nitrogen that they need to grow.

Now, of course, there's a lot of nitrogen, nitrogen gas, in our atmosphere, all right? But nitrogen gas is incredibly inert. You use nitrogen gas is an inert gas to basically stop things that are too reactive from reacting with the air in the lab, OK? Nitrogen gas as a very stable molecule is not at all useful to biology.

However, if we take this nitrogen gas and we reduce it-- that is we add a bunch of electrons-- what we end up with now is we end up with all of these reduced nitrogen groups, these amino groups that are incredibly useful for biology. These come up over and over again. These are the side chains of our amino acids. They're found on all the cofactors. They're forming the hydrogen bonds between our different nucleosides, OK?

And so enzymes work. Genetics happen. Hydrogen bonds form because of all of these nitrogen-containing compounds. But you need a way to get from this nitrogen gas down to this reduced nitrogen. And that is really, so-called nitrogen fixation, which is basically taking nitrogen gas and reducing it to be a compound like ammonia, which involves adding electrons, that ends up being one of the things, the magic of biology, that really makes life possible.

Now, you don't often think about nitrogen fixation very much, so obviously, we all know that photosynthesis is really central to what makes life happen. And we spent a lot of time talking about photosynthesis. Apart from photosynthesis, which, of course, takes the energy from the Sun and gives us a way to get reduced carbon that all life depends on-- nitrogen fixation is a way to take nitrogen gas from the atmosphere and turn it into ammonia, a useful form of nitrogen for life to use.

And what you may not realize is nitrogen fixation really only occurs in a select group of prokaryotic organisms. So these are really the unsung heroes of life, or at least the unsung heroes of metabolism. It's probably the most central topic to life that we're not going to spend a lot of time talking about in 705 simply because we don't have time. But it's basically redox chemistry to take nitrogen gas, add electrons to it, and end up with reduced nitrogen.

This is very energy intensive-- requires on the order of 16 ATP molecules. It's covered well in various textbooks if you're interested in reading about this further. But I really want you to appreciate that this is something that really has to happen, and it's really relatively few organisms that allow this to happen for all other life on the planet to be dependent on.

This is really why fertilizer ends up being the thing that you add to your garden. So in the wild, nature would set up symbiotic relationships with other organisms, with these nitrogen-fixing organisms, in order to get the nitrogen they can get. However, we can help that process along by giving fertilizer. If there's a lot of fertilizer runoff, you get algae blooms. That's really because all this nitrogen now gets dumped into the water. That ends up being rate-limiting, often, for photosynthetic organisms, like algae, to grow.

And so this ends up being a really important topic if you're interested in environmental science or if you're interested in agriculture. And it's at least something I want to make you aware of from this introductory course. All right. Now, for most organisms, we get, from either our diet or from the environment, fixed nitrogen. And the two forms of fixed nitrogen that we need to discuss is ammonia, as well as the conjugate base of ammonia, which is ammonium.

Now, fertilizer is, of course, very rich in ammonia. And animals that eat other organisms get lots of nitrogen from breakdown of proteins. And that is a source of ammonia or ammonium ions. And so photosynthetic organisms have the problem of getting these. Animals off and have the problem of, how do we get rid of this?

Now, if you've ever cleaned with ammonia, you know that it's pretty nasty. It smells bad. It's a cleaning agent. It's very toxic to lots of organisms at high enough levels.

And so animals really need strategies-- or, all life, really, cells need strategies about how they're going to handle and deal with these nitrogen-containing compounds, either to incorporate it or to get rid of it. Now, I'm going to spend a lot of time today talking about how animals who take in a lot of-- eat other organisms, take in a lot of nitrogen have to deal with the nitrogen excess. And it turns out that you can deal with the ammonia that's generated from the breakdown of your amino acids in lots of different ways.

And basically, there is no one solution that life uses that's universal for how you deal with the excess nitrogen. Really, different strategies have been involved incorporating the metabolism of amino acids and nucleic acids that fit the lifestyle of different creatures.

And so fish and other aquatic life really deals with nitrogen because they can just excrete ammonia directly. They live in the ocean-- lots of excess space into the ocean. Just take the ammonia. Send it off into the ocean. The large volume of water in the ocean means dealing with ammonia is not such a big problem-- literally pissing into the ocean to get rid of their excess ammonia, their nitrogen waste.

Now, terrestrial animals-- I'll just say terrestrial creatures-- have a different problem to deal with. And that is they can't tolerate the high concentrations of ammonia that would be necessary to just release it into the environment, so they need another solution. And of course, our solution is to excrete our excess nitrogen as urea.

So that's urea. This is why we make urine. And really, our solution to eliminate excess nitrogen is to concentrate that nitrogen, rather than excreting it as ammonia-- but to make urea, put it into our urine, and eliminate it through urination.

Now, not all animals make urea. Things like reptiles and birds, which-- turns out making urea requires access to lots of water. Reptiles don't always have access to lots of water. They live in dry climates.

Birds don't want to have to carry around all the extra water weight of dealing with urea. So instead of urea, it turns out that they excrete their nitrogen as uric acid. So what's uric acid?

So this here is uric acid. So uric acid is basically a purine. So it should look very similar to the adenine and guanine rings that you saw earlier in the class. And so they basically generate uric acid, which allows them to excrete more nitrogen with less water.

And so bird poop-- birds don't urinate-- the bird poop that you get, that slurry of material, is basically a mixture of solid waste and uric acid. And that's how birds eliminate their waste all in one go as a way to save some water as they deal with their excess nitrogen.

All right, the fact that different terrestrial creatures-- some, like us, make urea-- some make uric acid-- really deals with, shows that urea-- you will see today is from amino-acid metabolism-- uric acid is, of course, from purine, from purine metabolism-- we'll talk about that in the last lecture of the course-- they really illustrate how the metabolism of amino acids and nucleic acids is really about the metabolism of nitrogen and how these pathways are really set up in a way that allows exchange of ammonia with amino acids, as well as with nucleic acids, as the nitrogen can really flow between all of these different biological molecules. And along the way, well, this allows a way to excrete nitrogen. It also, obviously, involves ways to incorporate it and is really the pathway cells use to both make and break down these compounds. All right.

Now, as humans, we, of course, eat a diet that's high in protein-- higher in protein. Now the current fad is to eat high-protein diets. That's the latest trend that's healthy. And this involves lots of nitrogen excess, and so we have to, as humans, urinate several times a day because we need to excrete lots of nitrogen.

Now, that's really our amount of urea out. So that urea out, really, in a healthy person is balanced with the nitrogen in from the diet? Makes sense? If we are not growing and we eat a certain amount of protein, if we eat more protein, that means we need to excrete more nitrogen. If we eat less protein, then we need to excrete less nitrogen.

And so the nitrogen in and out should be balanced. Now the reality is that not all of our nitrogen is excreted as urea. Some of it ends up in the stool as other compounds. And so for a healthy person, nitrogen in is greater than nitrogen out in the urine, the urea. Nitrogen is really greater than nitrogen out. And this is what is referred to medically as positive nitrogen balance.

So if I collect all of your urine for 24 hours and keep track of all the food that you eat, and I measure all the nitrogen in the unit and all the nitrogen in the food you eat, there's going to be more taken in than I measure in the urine, that's positive nitrogen balance. And that is what is standard, healthy for most humans.

Now, if there's positive nitrogen balance, it means you can also have negative nitrogen balance, which really means that the amount of nitrogen in is less than the amount of nitrogen out. That is you are losing more nitrogen in the urine than you're taking in. Medically, this is a very bad thing.

When does this occur? Well, it occurs during periods of starvation. Make sense? If you're not eating any food, you still need to get energy from somewhere. You'll catabolize your glycogen, you'll catabolize your fat, but you'll also catabolize the amino acids in your muscle. Those amino acids will generate excess nitrogen, more nitrogen out than you've taken in the diet.

This also occurs during some illnesses-- famously, cancer, AIDS, sepsis, all of these really horrible conditions that some people-- can be very life threatening. Many of these lead to a phenomenon known as cachexia. So what's cachexia? You waste away, lose all of your lean body mass.

This is also basically a state of negative nitrogen balance. And why this is really, a current topic that lots of people are trying to figure out. But it's really a case where you are breaking down more of your muscle protein, more amino acids, and losing that nitrogen than you're taking in. And treatment of the underlying condition is really required for this to get better. All right.

Now, it turns out I can experimentally cause negative nitrogen balance in an animal if I remove certain amino acids from your diet. Why is that? Well, it's because prokaryotes, plants, yeast, lots of organisms, many organisms, can make all 20 of the amino acids that are required to build protein.

But it turns out that animals, including humans, cannot make all 20 amino acids. It's a quirk of our physiology. But it turns out some of the 20 amino acids that are needed to build protein are essential. That is they have to come from external sources.

So remember, there's lots of vitamins out there that we can't make. We have to get those from our diet. There's essential lipids. We talked about that last time again-- have to get those from our diet. And some of the amino acids that we need to live also have to come from an external source.

And in the 1950s, experiments actually done on humans, on medical students, actually found that if you take these essential amino acids out of the diet, what happens is that you can shift those medical students into a state of negative nitrogen balance. And that's because they're unable to synthesize protein. You're always synthesizing and breaking down protein. If you don't have the essential amino acids to resynthesize your protein, you now break down more protein than you synthesize, and you end up with negative nitrogen balance.

So what are these essential amino acids? Well, I post them here on this picture. And so here's the essential amino acid. They have to be obtained from nutrition. Here's the non-essential amino acid. These can be obtained from the outside, but we also have the pathways in order to make them.

If you count these up, you will see that there are nine essential amino acids and 11 non-essential amino acids. It turns out that two of the non-essential amino acids require essential amino acids to make, so they're sort of conditionally essential. From a trivia standpoint, I guess, the answer is that there's nine essential amino acids, and they are-- I'll use the one-letter abbreviation. So there's lysine, histidine, isoleucine, leucine, valine, threonine, tryptophan, phenylalanine, and methionine.

Now, there's two other ones. If you have enough phenylalanine, we can synthesize tyrosine. And if you have enough methionine, we can synthesize cystine. But it turns out that tyrosine and cystine because they require phenylalanine and methionine, for all intents and purposes, are also essential. So really, 11 of the 20 amino acids come from our diet, either directly or indirectly in the case of tyrosine and cystine.

Now, of course, our diets involve lots of proteins. We eat other animals, and so rarely is it an issue that we need these, which is probably how evolution was able to lose the ability to make them, because we take in lots of amino acids all the time anyway. And these are amino acids that are primarily important only for making proteins. But this also really illustrates that proteins are a large reservoir of the nitrogen we take in, and we just take it in premade already as these amino acids, even though we can synthesize the remaining nine.

All right, now of the remaining nine amino acids-- these are the ones that we can synthesize-- these are really largely ones about how we move nitrogen around through biological systems. We'll see that as we go through the rest of the lectures on the pathways involving amino-acid metabolism.

And where I want to start with this is how we're going to deal with ammonia, which is really, exchanged in and out of the biological molecules, mostly the two of the remaining amino acids. And those two are glutamate and glutamine. So I'll just remind you because this is important-- E is the one-letter abbreviation for glutamate. Q is the one letter abbreviation for glutamine. And glutamate and glutamine are really central to exchanging ammonia in and out of molecules.

Just to remind you what these look like-- so this is the alpha-keto acid, alpha-ketoglutarate, ketone, alpha to the carboxylic acid, alpha-keto acid, alpha-ketoglutarate. So remember, it differs from the amino acid glutamate. So exchange that alpha-keto group for the amino group. Now I have glutamate. And glutamine is, rather than carboxylic acid on the side chain, you add this second nitrogen group.

And so what you will see as we go through the lectures, that glutamate is really-- the major glutamate, alpha-ketoglutarate is really the major nitrogen donor and acceptor to get nitrogen in and out of other amino acids. In the last lecture, you'll see that glutamine is an important nitrogen donor. This nitrogen on the side chain ends up being important for getting nitrogen into nucleic acids.

Now, a really key enzyme to getting these nitrogen in and out is the one that catalyzes this step right here. This is catalyzed by an enzyme called glutamate dehydrogenase. And glutamate dehydrogenase really carries out the following reaction. There's alpha-ketoglutarate. So this can pick up an ammonia molecule.

OK. So with this intermediate. And now we have two electrons here as a hydride ion, which, of course, we're donating two electrons. That's why it's a dehydrogenase. It Involves nicotinamide cofactor. So these can come from either NADPH or NADH, generating NAD+ or NADP+.

And so reduce this molecule-- oxidized NADH, or NADPH, NAD+, or NADP-- and you get the amino acid glutamate. And so here's a reaction catalyzed by glutamate dehydrogenase that can convert the alpha-keto acid, alpha-ketoglutarate, into the amino acid glutamate. This reaction, which effectively is ammonia plus alpha-ketoglutarate plus NADH or NADPH can reversibility generate glutamate plus NAD+ or NADP+ plus water.

So this reaction, again, glutamate dehydrogenase-- very reversible reaction-- can use either NADH, or NADP, or NAD+, or NADP+. Either factor works, and it's a key reaction to convert the alpha-keto acid, alpha-ketoglutarate, with the amino acid glutamate and get ammonia in and out of the amino-acid pool.

All right, so I mentioned glutamine is the other major nitrogen carrier. And so how is this made? Well, you can make it. So if you start with glutamate, and you phosphorylate that glutamate with ATP-- so that's now a phospho acid on the side chain of glutamate. Lose the inorganic phosphate, and now we end up with glutamine-- glutamine.

All right, this is carried out by an enzyme called glutamine synthase. And it's basically another way to pick up ammonia.

This process can be reversed. So we can take glutamine. So I'm going to draw out the whole molecule. I'll just draw here the side chain. So this here would be the side chain of glutamine. Can just use water to remove the ammonia and regenerate glutamate carried out by an enzyme called glutaminase-- so glutamate to glutamine, glutamine to glutamate-- glutamine synthase-- glutaminase.

Notice, just like any other reaction or pathway we talked about, one direction is favorable. The other direction is not. So this direction requires energy input for it to work. ATP is used in glutamine synthase. Glutaminase doesn't require ATP because it's favorable-- makes sense with all the other stuff we talked about.

And so as animals, the way our tissues deal with nitrogen is that if we catabolize an amino acid in our tissue, we end up generating ammonia. That ammonia ends up either in glutamate via the glutamate hydrogenation reaction, or it involves in glutamine. And then we carry that nitrogen as glutamate or glutamine in our blood. It gets transported to our liver and kidneys, and our liver and kidney handle that excess nitrogen by, in our case, turning it into urea. Other organisms might turn it into something else in specialized tissues, where it can then be excreted, all right?

And again, it also ends up being that glutamate-- if we're going to make amino acids-- ends up being a good source of nitrogen. And glutamine, if we're going to make nucleic acids, end up being a good source of nitrogen. And so for catabolizing amino acids for energy, we can release ammonia via the glutamate dehydrogenase reaction moving in this direction, or we can incorporate ammonia by running in the opposite direction.

And so what you will see then is that the synthesis and breakdown, now focusing on amino acids, is really going to involve exchanging the nitrogen between alpha-keto acids and amino acids and glutamine and glutamate as we described for the malate-aspartate shuttle, OK? So let's just show an example of that. So this is what was involved in the malate-aspartate shuttle.

OK, so here's oxalacetate. So alpha ketone to the acid-- so alpha-keto acid. This here is the amino acid aspartate. Remember, in the malate-aspartate shuttle, we basically exchanged the amino group and aspartate to be glutamate and the alpha-keto acid, oxalacetate, for alpha-keto glutarate-- so alpha-keto acid, amino acid, alpha-keto acid, amino acid balanced on both sides. And nature can basically use this system to now get nitrogen in and out of systems, OK?

So take an amino acid alanine. It's related to the alpha-keto acid pyruvate. You can exchange that amino group onto alpha-keto glutarate, OK? That will generate glutamate.

You can use that glutamate. Transfer the amino group on to oxalacetate. That can generate aspartate. And so really, here, you have a system where you can use transfer of these amino acids between alpha-keto glutarate and glutamate and really, any alpha-- or sorry-- any amino acid and any alpha-keto acid, OK?

And so if we're going to catabolize an amino acid, say, alanine, give the nitrogen to alpha-ketoglutarate, now it ends up on glutamate-- that glutamate can go off in the blood. It can be used elsewhere to synthesize aspartate from oxaloacetate or some other amino acid from some other alpha-keto acid. Or you can just stay on glutamate, go here to the glutamate-dehydrogenase reaction and generate ammonia.

And so for catabolism of amino acids, the first step involves transferring the nitrogen to alpha-ketoglutarate, and that generates glutamate, as well as the alpha-keto acid, which can then be metabolized. If we're going to make an amino acid, we can start with the alpha-keto acid and transfer the nitrogen from glutamate. And that's a way, then, to make the amino acid. And so glutamate dehydrogenase lets you get ammonia in and out of the system, and then this basically transferring of nitrogen between alpha-keto acids and amino acids involving glutamate and alpha-ketoglutarate now allows you to move nitrogen around the system.

And so that's basically, in a nutshell, how you synthesize and break down many amino acids, although, of course, there are some exceptions. And so glutamine we just described. It's the glutamine synthase and the glutaminase reaction. Obviously, that's how you synthesize and break down glutamine relative to glutamate.

Asparagine is like glutamine. Remember, asparagine-- also, it's aspartate with an amino group on the side chain, just like glutamine. And so its metabolism is similar to glutamine.

Arginine-- arginine is a special amino acid with three nitrogen molecules. We'll discuss that at length in a little bit.

Prolene-- if you look up the structure of prolene, what you'll find is that it's glutamate that's been cyclized, then reduced. And so prolene is also a little bit different. You make glutamate, cyclize it, and reduce it. That's how you get prolene.

And then glycine, it turns out, is generated from serine. And we'll discuss that at length in the next lecture. But for the other-- most of the other amino acids, reactions really involve interconversion between the alpha-keto acid and the amino acid and glutamine-glutamate via series of reactions referred to as transamination. OK and so transamination is really these reactions that allow this swapping of nitrogen between amino acids and alpha-keto acids and alpha-ketoglutarate and glutamate.

Some of these are very famous. Many of you want to go to medical school. You're going to do liver-function tests, where you measure levels of ALT and AST. These are the liver-function enzymes, or the transaminases, all right? They're basically the transaminases that catalyze the exchange in the case of ALT between alanine pyruvate and glutamate alpha-ketoglutarate.

AST is the transaminase that catalyzes the exchange between aspartate oxalacetate and glutamine alpha-ketoglutarate-- very active, highly reversible enzymes, very abundant in the liver. The liver is important to get nitrogen in and out of across different amino acids-- high levels in the liver cells-- so when the liver is damaged, they leak out, and they measure in the blood, and that's a sign of liver damage.

And so again, just to drive this point home, so you have alpha-ketoglutarate. Alpha-ketoglutarate can be converted to glutamate. This is the glutamate-dehydrogenase reaction, so it involves a redox step with NADH or NADPH. That allows you to interconvert the ammonia, basically, take nitrogen in and out of the system as ammonia. And then once it's in the system, now you can do transamination to move the nitrogen between glutamate alpha-ketoglutarate with any other alpha-keto acid and amino acid, all right?

And so if you're going to catalyze amino acids, you go in that direction. And you're a fish-- you go this direction, release it as ammonia. Or you can come in this direction, and that is anabolism. That allows you to build amino acids, all right?

OK. So these transamination reactions then become very important, and we need to discuss how they work. So Transamination itself requires a cofactor. This cofactor is called pyridoxal phosphate, OK? It's derived from a vitamin, like many cofactors. In this case, it's vitamin B6, also referred to as pyridoxine, vitamin B6. Vitamin B6 looks like this.

OK, so that's pyridoxine. That's vitamin B6. Turns out we eat vitamin B6. And the active form in the cell is this molecule, where this alcohol has been oxidized to the aldehyde. And this Coke factor is referred to as pyridoxal phosphate and abbreviated PLP, for Pyridoxal Phosphate. All right.

All right, now let's go over now pyridoxal phosphate allows these transamination reactions to occur. So let's start off here with just some generic amino acid, OK? And so the transaminase has-- so here's pyridoxal phosphate. I'm not going to draw the whole molecule-- just a skeleton of the pyridoxal phosphate.

So it reacts with the amino acid. OK? So that's that middle part here of the molecule. OK? Generate the shift base. Just draw the top part here.

OK, so here now we generate the alpha-keto acid. And this alternative version of pyridoxal phosphate called pyridoxamine, which is also abbreviated PMP-- and so basically, I've exchanged the amino acid, transferring the nitrogen onto PLP to make PMP and generating the alpha-keto acid.

That PMP can now react with some other alpha-keto acid. So here's our PMP. And basically, if I just reverse all of those exact same steps that I just did-- so I'll do, for the sake of brevity here, I'll do two steps in one. OK, so I broke this up here into two different steps before, but to save me some drawing, I'm just going to do one here.

And all I did is just run the same mechanism in the opposite direction. And now I've taken my alpha-keto acid, got the nitrogen from PMP, regenerated now a new amino acid, as well as-- I'm not going to draw it all out, but regenerated pyridoxal phosphate.

And so pyridoxal phosphate ends up being this really useful cofactor that enables this transamination reaction that is this interconversion between an amino acid and alpha-keto acid and another amino acid or alpha-keto acid. And so pyridoxal phosphate is a co-factor that also useful for other amino-acid-containing reactions-- basically enables this sort of chemistry that enables nitrogen to-- this sort of chemistry to facilitate reactions with amino acids.

We will see in the next lecture that we will use pyridoxal phosphate in a slightly different way to convert serine to glycine plus a one-carbon unit that we can use for something else. I don't have time to go into them in this introductory course, but pyridoxal phosphate is also involved in other amino-acid reactions. So last time, we discussed ethanolamine. And remember, ethanolamine is an alcohol that's found in phospholipids, phosphatidylethanolamine.

Serine is used to generate ethanolamine. And pyridoxal phosphate basically, enables decarboxylation of serine to generate ethanolamine. Serine can also be turned into pyruvate and ammonia. It's an alternative way to generate ammonia. That also involves pyridoxal phosphate. And if you're interested, you can look up these reactions and their mechanisms and see how pyridoxal phosphate is repurposed for those reactions, all right?

But for today's purposes, this is what pyridoxal phosphate is. This is how it allows transamination. And so now it should be very clear how it is that fish can eat a bunch of protein-containing food and excrete nitrogen into the ocean, all right? And that is they can take whatever, some amino acid. They can do a pyridoxal-phosphate-containing transamination reaction and generate the alpha-keto acid with the nitrogen going on to alpha-ketoglutarate to generate glutamate.

This alpha-keto acid can now be oxidized. That's favorable. That can be used to generate ATP and allow the fish to get the energy it needs to swim.

That glutamate can now basically carry out the glutamate-dehydrogenase reaction, generating ammonia, regenerating alpha-ketoglutarate to accept the next nitrogen to allow oxidation of the carbon backbone in the amino acid. And the ammonia can float off into the ocean where it doesn't hurt anybody and be fertilizer for some algae. All right? And so there is a way to take catabolism of amino acids, get energy, and excrete the nitrogen as ammonia, basically involving transamination, which requires pyridoxal phosphate and glutamate dehydrogenase. All right.

Now, it turns out tadpoles do the same thing because they live in water. But when a tadpole becomes a frog or if you're a mammal, you don't do this anymore because now you don't have infinite water to excrete into. And so instead, as I said earlier, we're going to generate urea as a way to excrete nitrogen. And of course, I mentioned earlier-- I'm not going to draw it again, but birds and reptiles-- because you'll see making urea requires a lot of water-- you can save even that water by making uric acid appearing instead.

Now, how you make purines will be discussed in the last lecture. You'll see how you make uric acid there. But for now, I want to discuss how we generate urea as a way-- an alternative way to deal with amino-acid metabolism and excrete nitrogen.

Turns out that as we discuss how you synthesize urea also discusses how we're going to make and break down arginine, one of those exception amino acids. And you'll see why arginine and urea are really tied to metabolism in the way they are. And so let me just remind you what arginine looks like.

OK? So this here is the amino acid arginine, all right? I'm going to redraw part of this side chain here in a different color. OK? And I did it that way because I want to show you how this is related to urea?

So if I take water-- taking off of that end of the arginine molecule generates urea, all right? And so what we're left with after we do that is this other amino acid, which is not found in protein but is found in metabolism. And it is an amino acid called ornithine.

And so basically, if you break down arginine into ornithine, you generate urea. And so it should be clear that if I can now create a cycle where I take ornithine, pick up a couple of nitrogen and regenerate arginine, now I have a way to basically, produce urea because then I can cycle arginine to ornithine, make urea, pick up two more nitrogen, regenerate arginine, and that's a way to generate urea.

Now, this series of reactions occurs via cycle called the urea cycle. And the urea cycle was also described by our old friend, Hans Krebs. So remember, Hans Krebs described the TCA cycle. Turns out the urea cycle was described by Hans Krebs before the TCA cycle and is really the first cycle described. And so part of the inspiration for the TCA cycle came because Hans Krebs already had cycles on the brain from describing their urea cycle.

Now, it is a common misconception that all cells do the urea cycle. That is absolutely not true. Lots of cells carry out reactions that involve the urea cycle because it's also involved in arginine metabolism, all right? But the urea cycle, as we're going to describe it, is really, at least in us, selective for our livers and our kidneys because this is really the series of reactions that we run to net-generate urea, which ends up in our urine. OK? And so remember, it would be nitrogen transported to the kidneys or the liver via glutamate or glutamine and then and end up ultimately, entering this urea cycle to generate urea.

Now, this involves reactions that will occur in multiple compartments in the cell. And so most of it happens on the cytosol, but there's one reaction that occurs in the mitochondria. And so a general overview is as follows, OK?

So I just showed you how you can start from the amino acid arginine and generate the non-proteinogenic amino acid ornithine, all right? And this will involve production of urea, all right? That reaction occurs in the cytosol.

But the next reaction, which is ornithine picking up a CO_2, as well as or anything picking up an ammonia to generate another non-proteinogenic amino acid called citrulline-- this reaction occurs in the mitochondria. And then that citrulline will pick up another nitrogen-- show you how it does this in a minute-- to make a molecule called argininosuccinate. And then that argininosuccinate will be turned back into arginine. And so a four-step cycle-- this reaction-- ornithine to citrulline happening in the mitochondria and then regenerating the arginine from citrulline in two steps happening in the cytosol and then the arginine-backed ornithine in the cytosol to generate urea. All right.

Now let's go through the details of how this works. And we're going to start here and the mitochondria at this ornithine to citrulline reaction.

So it turns out the first part of this is generating a molecule referred to as carbamoyl phosphate. I'll write that out in a second. And so this comes from CO2, which, of course, is in equilibrium and cells with bicarbonate, OK? So there's bicarbonate.

Just like we saw when we did carboxylation reactions, if I phosphrylate the bicarbonate with ATP, this molecule here then would react with biotin, and that was a way I did carboxylation reactions. Well, it turns out that rather than react with the nitrogen on biotin, I can also react with ammonia. That generates this carbamic-acid intermediate, which can then be phosphorylated with ATP, again, to generate this molecule, which is called carbamoyl phosphate. OK, so carbamoyl phosphate.

It turns out this whole step-- two ATPs taking CO2 and ammonia and generating carbamoyl phosphate is carried out by a single enzyme. This carbamoyl phosphate will then react with an ornithine molecule. So here's ornithine. OK. And that will generate this molecule, which is the amino acid citrulline, ornithine and citrulline being the two major amino acids not found in protein.

All right, now this citrulline, then, in the cytosol can undergo a reaction with ATP. And it's easier to see if I rearrange this part here of the side chain of citrulline. So I'm just here going to draw the side chain.

So it turns out this will react with ATP. So I'm just going to draw ATP here in this stylized way. That's going to release pyrophosphate, which can be, of course, pulled forward by cleaving the pyrophosphate into two inorganic-phosphate molecules.

And so now you have citrulline with an AMP on it. And that can react with an aspartate, the amino acid aspartate-- so this is the amino acid aspartate-- losing the AMP to generate this intermediate-- this intermediate, which is called argininosuccinate.

So argininosuccinate-- why is it called that? Because this is a succinate molecule here. And this part here is an arginine molecule. And now what can happen next?

Oh, by the way, this reaction here from basically, adding citrulline to argininosuccinate-- it's carried out by one of my favorite enzyme names. It's called Argininosuccinate Synthase, abbreviated ASS. So the enzyme is the ASS enzyme-- A-S-S-- Argininosuccinate Synthase-- to turn citrulline into argininosuccinate.

Once you have argininosuccinate, you can now carry out that reaction, which will generate two things. From the purple half here, you will now end up with this molecule, which is fumarate from the TCA cycle, as well as I've regenerated one of the most tedious amino acids to draw, which is arginine. So here, we have our amino acid arginine.

OK? And so that fumarate now needs to be, of course, turned back into aspartate. You know how to do that. I can take fumarate, turn it into malate. That's adding water. That's the TCA cycle Then malate to oxalacetate-- that's NAD to NADH. And then aspartate can be transaminated into aspartate.

And you can basically see how I can run that you urea cycle as a way to excrete nitrogen one, from carbamoyl phosphate, one from aspartate to generate urea. And so this complete cycle is actually a few cycles put together, sometimes referred to as Krebs bicycle, or Krebs tricycle. And I'll just draw it out here.

So if you start with argininosuccinate here in the middle, that argininosuccinate will generate fumarate and arginine, OK? Arginine into ornithine will allow generation of urea. Ornithine can be turned into citrulline, picking up a carbamoyl phosphate, which, of course, is from CO2 plus ammonia. That citrulline and aspartate come together to give you argininosuccinate. And then that fumarate can run via the TCA cycle to generate malate. So that's water.

Malate can be used to generate oxalacetate. So that is, of course-- OK. That oxalacetate can be used to generate aspartate. That's a transamination reaction.

So that transamination reaction [INAUDIBLE] glutamate to alpha-ketoglutarate and then glutamate to alpha-ketoglutarate. Glutamate-dehydrogenase reaction can bring the nitrogen in.

And so this here complete cycle-- what you'll see is actually balanced with respect to both electrons because of the glutamate-dehydrogenase reaction and the malate-oxalacetate direction, as well as all the nitrogen, really allows you to take nitrogen as ammonia, put it in the system as aspartate, use that to combine with citrulline to make argininosuccinate. Go to arginine, make urea with the other nitrogens coming from carbamoyl phosphate to generate the citrulline.

And so the ammonia that's brought to the liver and the kidneys via glutamate or glutamine can then be incorporated into aspartame and carbamoyl phosphate and ultimately, excreted as ammonia. You can see that there's lots of ATP that's required here. You need two ATP here to generate carbamoyl phosphate. And you need another two ATP here at the argininosuccinate synthase step to generate argininosuccinate. And you also need CO2, but the net effect is that you generate urea.

OK, I know that the urea cycle is complicated. I will start out next time running through another review of it just to make sure you have it clear. Thank you.