Good morning. Good morning, yes, thank you. Well, we were coming to the end of the term rather soon. That's sad.

And, what I'd like to do today is, picking up on some of the stuff that Bob has been doing, begin to show how the things we've learned about understanding molecular biology, biochemistry, genetics, all come together to help us treat disease. That is after all the point of all this.

Bob spoke about this with respect to cancer. Today my goal is to talk about this with respect to heart disease. We've got about 50 minutes or so, and I'd like to see if we could solve heart disease by the end of the period. That seems like a reasonable goal.

And so, I would like you, by the end of today's class, to design a therapy to cure most people or at least to prevent a couple million heart attacks, all right? So, that's our goal.

So, we'd better get to work if we're going to accomplish that in the allotted time. So, first off, you need to know a little something about heart disease.

The heart: that's obviously an important component of understanding heart disease. What's the heart do?

The heart pumps blood, and to and from tissues providing nutrients, hormones, removing waste products, cells, for example, it pumps around red blood cells and white blood cells and things like that, and of course oxygen in the blood. These are incredibly important things that your heart does. If your heart should stop doing it for even relatively brief periods of time, it's very bad news.

This is extremely serious not to have your heart pumping, as you all know. One of the ways in which you run into trouble with your heart pumping is if the vessels that carry blood away from the heart to the periphery arteries become clogged.

Just simple plumbing problem here: if they become clogged and the arterial wall gets build ups here, we have what is called arteriosclerosis. And, it can lead eventually to nearly complete blockade of a vessel. And if that vessel, for example, were to be supplying something important like, for example, the blood supply to the heart muscles itself, that would be extremely bad, all right, because then your heart muscles wouldn't have oxygen, and they would quickly die.

Other problems can happen here too, where you could have insufficient blood supply to the brain. What happens when your brain doesn't get enough oxygen: stroke. So, we have many, many of these issues. So, if it's the brain: stroke. If the tissue that doesn't get enough oxygen is the heart, you've got enough heart attack.

And so, we want to prevent this process of the buildup of plaques in the arteries. And these plaques are made up

of complex mixtures of proteins, lipids, and cholesterol, and surely by now you all know that cholesterol was this evil molecule, and it will indeed be the villain of today's story.

So, that's the basic plan there. I'll draw your basic plumbing diagram here just so you have it. We've got a heart here. This is your heart, somewhat simplified picture here. The heart pumps blood that goes out to the body, to the heart itself, to the brain, and of course this before it's doing it is receiving oxygenated blood from the lungs. So, it's really all of these places that we're trying to keep flowing. The number of heart attacks, deaths from heart attacks, and other cardiovascular disease is extraordinarily high. It's on the order of 1.

million deaths per year. By contrast, number of deaths from cancer is about 600, 00 or so, something like that.

And, this has been coming down a little bit for cardiovascular disease partly because of some of the things we'll talk about today.

But it still is a leading killer of adults in this country, and indeed the leading killer of adults in this country, although there are projections that cancer may take over in that role.

Heart disease is incredibly important. So, if we're going to try to understand how we're going to prevent these plaques, and I've told you already, and you know from the media that these plaques have lots of cholesterol in them, and that you all know that high cholesterol is bad, how do we know that high cholesterol is bad?

Correlation. Epidemiological correlation is one, and as we will see today, there are some other, more direct results coming out of genetics that point to this as well.

What is cholesterol? Let's talk about cholesterol.

So, this is the structure of cholesterol.

There we go. That's cholesterol. It's a very complex, interesting molecule here with lots of rings. It is a waxy substance. If you were to look at a test tube with a lot of cholesterol in it, it would look like wax. And, it is extremely hydrophobic. It will not dissolve in water. So, if cholesterol is such an evil molecule as you all know from the news media, why do we have this evil molecule? Sorry? Well, because it's absolutely essential for life. I mean, despite it's rep as an evil molecule, it's extraordinarily important. The uses of cholesterol are many. One you've already said. It plays a structural role in cell membranes, the plasma membrane. This is not a big player role in the cell membrane. But about half of all the lipids in the cell membrane are cholesterol. It's a huge component. Cholesterol molecules, because of their funny shape, helps stiffen membranes and strengthen membranes. So they strengthen and stiffen the membrane. Also, not only are half of the lipids in your cell membranes cholesterol, half of the cholesterol in your body is in the cell membranes. So, that's the major location. They're also used,

these complex molecules, as precursors for the synthesis of steroid hormones.

Steroid hormones, of course, also are evil these days.

Those of you who checked the New York Times today saw that Jason Giambi admitted using steroids to a grand jury. But steroids are also, notwithstanding those kinds of things, good for you.

What are some important steroids in your bodies: testosterone, estrogen, glucocorticoids, all of these things are made from cholesterol. It was a precursor.

And if you look at the structure of steroid hormones, you recognize that this coupled ring structure here is very similar to what's in them. And indeed, they're derivatized from them. It's also a precursor for the synthesis of vitamin D.

And, it is a precursor for the synthesis of bile acids.

When your body takes in triglycerides in your food supply and you need to transport fats like triglycerides across your intestine, they need to be emulsified. The way that triglycerides are emulsified are with bile acids. So, you secrete bile acids into your digestive tract. It helps emulsify fats and helps you take them up. So, cholesterol plays a crucial structural role, a biochemical role with regard to hormones, with regard to vitamin D synthesis, and with regard to bile acid synthesis. So, cholesterol was a good thing, all right. Now, if cholesterol is so extraordinarily hydrophobic, how is it that cholesterol gets around the body? It's almost entirely non-polar.

It doesn't dissolve in water. It has one little hydroxyl there.

It's not going to help a lot. Yep? Well, the first thing actually is it's chemically modified to make it a little hydrophilic and then it does bind to hydrophilic proteins and particles that help get it around. So, the first thing that happens, to be able to, even just to store cholesterol in any useful form, it's not stored as cholesterol because it's so waxy.

It would just collapse as a waxy deposit. What happens is it is stored and transported as cholesterol ester.

A cholesterol ester, or CE, and its esterified by adding to it, here's my cholesterol again.

I'll be even less, there we go.

This hydroxyl here is used now for a fatty acid linkage.

And with this fatty acid attached to the cholesterol, you have a cholesterol ester. And this is somewhat more soluble.

All right, where do you get your cholesterol from?

Diet. Do we eat cholesterol? Butter's got cholesterol. What else has got cholesterol? Eggs, a lot of cholesterol.

So, we eat cholesterol. So, let's get our sources of cholesterol: number one, diet. And, sources, eggs, butter, etc. What else beyond diet?

Yeah? Your body actually makes it. Your own endogenous synthesis. And you would imagine that this is pretty important because cholesterol, being half of all plasma membranes, you can't just count on cholesterol being sufficiently there in your diet.

So, your body also synthesizes cholesterol. The synthesis of cholesterol is a thing of beauty. It starts with an incredibly simple molecule, acetic acid, right? And it goes through many steps and becomes cholesterol, which I will summarize here as many steps, OK? We'll come back and talk a moment about a few of those steps. But it's one of these real triumphs of biochemistry; the people have worked out the whole pathway for cholesterol biosynthesis. But it's not, I think, necessary to remember all of the steps there. But it is quite remarkable to go from such an extremely simple molecule like acetic acid all the way to cholesterol. And the fact that people worked all this out was a great achievement. All right, so those are some of the things you need. Then, carrying on here, we've got cholesterol coming in by diet. We're synthesizing cholesterol. We're making cholesterol esters.

We've got to get them around the body. So now, we're going to take these cholesterol esters and we're going to package them up and send them off. So, you were saying that proteins would be used. Hydrophilic proteins might be used.

And indeed, that is the case. Lipoproteins and lipoprotein particles, lipo being fat, of course, are used to transport cholesterol and actually triglycerins too.

They are transported in particles that look roughly like this.

They have a monolayer of phospholipids with some protein stuck in this monolayer of phospholipid.

And, inside is where these cholesterol esters go.

So, actually this is where cholesterols go unesterified.

So, cholesterol goes in here. In the cell, we want it esterified.

When it's in the package, it's unesterified. So we have a monolayer of phospholipids. We've got some protein stuck in that monolayer, and it's a very little particle.

Now, these particles come in different flavors.

These are very creative names: low-density lipoprotein particles, or LDL. There are high density lipoprotein particles, HDL, and very low density lipoprotein particles, VLDL, and some other things called kilomicrons. Now, you can imagine that these names were assigned based on not so much information, just based on density, right? Somebody was purifying lipoprotein particles and said, well, there are some that are high density, low density, oh, and you just discovered some very low density ones.

And this is not a highly informative description of these particles, right? So, people later worked out that these particles were really quite different, and particularly the proteins that are in them, and those proteins turn out to have important addressing roles in sending these particles to different places. The ones that I'll be interested in today are the LDL particles. The LDL particles have a particular protein in them that is called apoprotein B-100. It doesn't matter, but that's the particular targeting protein there that's in that.

And these particles are very large. They are about two and a half million Daltons, about 220 angstroms in size, and each of them contains about 1, 00 cholesterols.

That's a description of these LDL particles, OK?

So, cholesterol, if it's going to be transported in the blood, gets packaged up into LDL particles, and it gets sent off to cells. How does cholesterol get taken up by cells from these LDL particles? How is that cell going to take up an LDL particle: a receptor, right? It stands to reason that there's going to be a receptor that's going to recognize probably the protein on the surface of this thing that'll recognize that and internalize this particle. Now, this stands to reason to us to you guys because you guys are highly sophisticated about all this.

But how was it that people came to know this, to find these receptors?

Well here is a little bit of an interesting story about how, not so long ago, when people didn't have all the tools in molecular biology and all this? And very few of these cellular receptors were known. Two young medical students began studying a fascinating condition. The young medical students were named Joe Goldstein and Mike Brown. And in fact, at least early in their careers they were working here in Boston. So, they studied a fascinating condition called familial hypercholesterolemia.

What does hypercholesterolemia mean? Cholesterol, hyper, a lot of cholesterol, emia, in the blood, right?

So, a lot of cholesterol in the blood was this condition.

And it was characterized, as you might guess, by the fact that patients had a lot of cholesterol in the blood and

that it was familial, meaning what? It transmitted in families.

In fact, it transmitted in families like a Mendelian trait, and it transmitted as an autosomal co-dominant trait.

In particular, if we looked at most individuals in the population, which we'll assume have genotype plus over plus, and we look at their cholesterol levels, what we find is maybe they have 150 mg per desalude. Now, some people have higher cholesterols than that, but I'm going to take that as an average. Individuals who, by virtue of their genetics, appear to be FH over plus heterozygotes would have cholesterols more like 300 mg per desalude, or about double the normal level. And, individuals who are FH homozygotes, FH over FH based on the pedigree analysis here would have greater than 600 milligrams per desalude.

In terms of heart attacks, normal individuals would have heart attacks at the normal age. That doesn't say anything, does it, because the normal individuals, the age at which they have heart attacks is defined as the normal age. But, what you will know that's striking is that individuals who are heterozygotes would tend to have heart attacks 10-20 years earlier than normal age. And, individuals who are homozygotes would tend to have heart attacks below the age of 20.

So this might be heart attacks when you're 60. This might be heart attacks in your 40s and 50s, and this might be heart attacks in your 20s or teens. In addition, some of these individuals have very big cholesterol deposits and things like that. So, this was a very striking phenotype: simple Mendelian trait, autosomal co-dominant, and to see teenagers or younger, kids under the age of ten with massively occluded vessels, and serious heart disease, and dying of heart attacks was very striking.

So, Brown and Goldstein decided that if we wanted to understand heart disease in the general population, we should understand heart disease in patients with familial hypercholesterolemia, particularly the homozygotes. Now, I note that this is about one per one million individuals. And you could make a pretty strong case that Brown and Goldstein are out of their minds trying to study familial hypercholesterolemia at a frequency of one in a million and try to imagine that that's going to tell them about heart disease in the general population. All right, and people made that case to them and said, what are you doing? Let's see, if this is P2, that means the frequency of the, Q2, the allele is one in a thousand in the population, right?

If one in a million people are homozygotes, the allele is one in 1, 00. And so, the heterozygote should be about one in 500.

Well, OK, so one in 500's not a terrible, it's not a small number.

One in 500 people are heterozygotes for FH. That's maybe a little better, but still not even a percent. It's a fifth of a percent of the population are heterozygotes for FH. It's still something of a gamble to imagine that by studying

this relatively rare disease we're going to learn about stuff in the general population.

But, Brown and Goldstein felt strongly that they would.

And they did. They wanted to know, what was the problem with these individuals? Did they have problems synthesizing their LDL?

Did they have problems degrading LDL? Why was there so much LDL cholesterol in the blood stream? Maybe they didn't take up the LDL from the blood stream. What was wrong? And so, they studied just these individuals.

And what they found, to make an interesting and long story short was that when they studied the binding of radioactive LDL particles to cells from these patients, they found that the homozygotes were virtually unable to take up LDL particles.

There was very low uptake of LDL particles. They also found that the heterozygotes had only about half the normal uptake of LDL particles.

What's your hypothesis about what the problem is? Sorry?

Well, it's with the uptake, and what do you think the genetic basis of this is? Yep? Well, let's see, if there's zero in the homozygote, half the level in the heterozygote, could be the receptor. And what could be the problem with receptor?

Mutation of the receptor gene. What if homozygotes or FH had a mutation that abolished the receptor?

OK, that turned out to be the case. Was the FH was due to mutations in the LDL receptor on cells? And indeed, until this point, the LDL receptor hadn't been characterized on cells, but by virtue of Brown and Goldstein demonstrating that when they did radioactive labeled LDL uptake assays, and they found that FH homozygotes had no uptake, virtually no uptake, and that the heterozygotes had half the normal level, and that the wild type individuals, plus over plus, had the normal level, they inferred that the gene product that was mutant in these individuals encoded the LDL receptor.

And they proceeded to clone this gene product, and determined that the gene product encoded a protein that sat on the cell surface.

It had a cytoplasmic tale. It had an extracellular tale, and what it did was it bound to the apo B-100 protein on the LDL particle. When it bound the cell made a little pit, and in this coded pit, it came and internalized the LDL receptor, carrying with it the LDL particle. And then, this then went into the cell. The LDL particle was then degraded, releasing cholesterol that could be used by the cell.

And the receptor itself got recycled back to the surface to work another day. And this is quite a general mechanism that is used there by a lot of trafficking receptors like this that grab things from the cell surface, bring them into the cell, release something into a vesicle, and then go back onto the surface there, and that the problem was that patients FH did not have functional LDL receptors.

Now, this pointed out, LDL receptors were very important here because cells needed to take up cholesterol from the blood stream, although they could make some of their own cholesterol.

But one of the most important places where cholesterol was sequestered and taken up from the blood stream was the liver.

It turns out that the biggest problem for these patients with familial hypercholesterolemia was that the liver is supposed to be taking up large amounts of LDL to clear the blood stream and keep the levels of LDL at the desirable amount. So, the liver normally is responsible for taking up clearing about 75% of LDL from the blood.

If someone has, and other cells are responsible for the rest, non-liver cells take up about 25% of the LDL.

Suppose somebody has half the level of LDL receptors, they will take up half as much of these LDL particles, and the average level in the blood would be much higher, about twofold higher. Suppose somebody has no LDL receptors. Well, then the LDL receptor pathway is not going to take up these particles, and they're going to have outrageously high levels of LDL. Other mechanisms will kick in and slightly prevent it from going to infinity, of course, because there are other ways things get cleared out.

But they get huge levels of LDL because they have no such receptors. So, this is the major reason that there's so much LDL cholesterol particles in the blood in these patients who are FH homozygotes. And then, FH heterozygotes have a lot. And, well, what are we going to do about it?

How do you solve a problem like this? The liver is not doing its job. Now, I should note, the liver does one other thing.

the liver not only is the major source of taking cholesterol, but it's the major source of producing LDL cholesterol as well.

The liver produces, I remember every cell is able to synthesize cholesterol, but most of the cholesterol in the body is synthesized from the liver and put out. And so, here we have the liver is a source of cholesterol, and it's in this disease not acting as an appropriate sake for a cholesterol. It ought to be sucking up cholesterol and maintaining a balance of producing cholesterol and soaking it back up there.

We've got a real problem. Well, we need to know one more fact, and then we can solve the disease. Well, we're not going to solve the disease, but we'll do the best we can here. So, cholesterol synthesis, I just want to tell you one more fact about it and then toss you the problem. Cholesterol synthesis, I said, was acetate, acetic acid, goes to stuff, and let me tell you just a little bit more about it.

It goes to acetyl CO-A, which goes to HMG CO-A, which goes to mevalonate, which goes on to make cholesterol, and that the key committed step of cholesterol synthesis is carried out by an enzyme called HMG CO-A reductase, OK?

Now you have all the facts. We know we've got these particles that contain cholesterol. We understand that the liver makes cholesterol, that you get cholesterol from your diet.

The liver makes cholesterol. It takes up cholesterol. We have some problems with its uptake of cholesterol. Let's get to work and design a therapy. How are we going to do this?

So, we've got patients designing a rational therapy.

OK, here's your digestive tract.

You've got some liver here it's going to take up by means of diet. It's going to synthesize cholesterol.

It's going to use cholesterol to make bile acids.

Those bile acids are going to help bring back fats because it's going to emulsify the fats. And so, the bile acids get recycled.

It's going to put out cholesterol to the body, and LDL particles are going to get internalized into the liver. So, we have our LDLs.

OK, so has everybody got the action? You take up a cholesterol, and fats, and things like that through our diet.

You've got some cholesterol in our body. We synthesize cholesterol from acetic acid. We have this pathway here.

We use cholesterol to make bile acids.

We take up cholesterol from the blood stream, and all of these things together working as a system control how much cholesterol is in your body, and most importantly, how much cholesterol's in your blood stream in the form of LDL particles. OK, we have a patient. Maybe it's a patient with FH homozygosity. But let's start easier. Let's start with a patient who's a heterozygote for FH.

What's our first advice? Eat well, get plenty of exercise: this is always good advice. So, plan one, so strategy number one: diet, dietary reduction of cholesterol intake.

Eat less cholesterol. It's a good bit of advice.

Stop eating eggs, whatever, you have a serious condition, don't eat so much better. Does it do much to reduce LDL levels?

It turns out, not much. Why doesn't it do much?

What if I reduce dramatically my intake of cholesterol?

Well, it only makes about a 10% reduction in LDL levels, which is not enough to get close to normal. Why? Well, it turns out your body, number one, gets more efficient at taking up cholesterol from your diet. So, you have some cholesterol there, and if you're eating less, it takes up with higher efficiency the cholesterol that's there. Your body's good at doing things like that. It also starts synthesizing cholesterol.

Don't have enough cholesterol? We'll make more cholesterol. So, the liver will make more cholesterol, put cholesterol out into the blood stream, and these guys can't take up the cholesterol with the LDL receptor as well, and it clogs it up again.

So in the end, between more efficient uptake of what is in the diet and greater synthesis, we've got to complex the human system with feedback, and all you've tried to do is affect one variable, dietary intake. And the system regulates so that you haven't made a very big dent in the problem. All right, next strategy, let's try to deplete some cholesterol from the body.

If we could get some cholesterol out of the body by some pathway, we might be able to decrease the overall levels of cholesterol.

So, where do we have access to a cholesterol product here?

In the digestive system, we have bile acids. Got any ideas of what we could do about the bile acids? Suppose we could somehow deplete your bile acids while they're in your digestive tract.

Then your body would not be able to recycle those bile acids, but would have to make more bile acids. And it would be a sink for cholesterol, right? It would start having to use up more cholesterol to produce enough bile acids because it would have to use up more cholesterol. The liver might have to work harder to get cholesterol, you know, synthesizing cholesterol, but it would also probably up regulate its LDL receptors to try to draw in more cholesterol from the blood stream.

So, this was the clever idea. Let's try to get ride of bile acids, or not completely rid of them, but let's try to complete bile acids.

Therefore, the liver is going to not be able to reuse them.

It's going to have to make more bile acids. It's going to need cholesterol. And so it's going to up regulate the gene for LDL receptors and draw in more from the blood stream. It turns out that you can feed people bile acid binding resins. It's perfectly fine.

Just eat them, and you can give people bile acid binding resins.

So, strategy number two, and what they will do is then they will, in their feces, eliminate some fraction of the bile acids and as a result they will be able to get rid of some of their cholesterol, and they will be able to decrease their overall cholesterol levels. Does this work?

It does, and you can get maybe a 20-25% reduction. But what's the problem? You can't completely get rid of them, right, because they're necessary. So, we'll be able to get rid of some bile acids. That's really the problem is, see, we're sitting here saying so cleverly we're going to feed the body less cholesterol. We're going to draw cholesterol out of the body by removing bile acids, and force the liver to take up more cholesterol from the blood stream, right?

And hopefully it'll help regulate the gene. And it does help regulate the gene. When starved for cholesterol, cells up regulate their LDL receptor gene and make more LDL receptors. That works.

But we've forgotten one aspect of the system, and that was the liver has another option, which was synthesize its own cholesterol. So, we've got a complex system with multiple feedbacks. We've affected it at the level of diet. We've affected at the level here of drawing stuff out.

We've managed to get some up regulation the LDL receptor gene.

But it's not enough because the liver's choosing to make more cholesterol through its endogenous synthesis pathway.

So, let's keep going. What do you recommend?

Yes? Ooh, ooh, I like that. What do you think will happen then?

So, I won't make as much cholesterol. What's the liver going to have to do then? And it will up regulate its LDL

receptors to do that, take up more cholesterol from the blood stream. So now, we back the liver into a corner, right? It needs more cholesterol.

We're going to inhibit the synthesis of cholesterol, and therefore it's going to go to its second source, which is uptake from the blood, and it's going to induce more LDL reception. Everybody got your plan? So, let's see.

Strategy would be inhibit cholesterol synthesis.

This is in addition also inhibit cholesterol synthesis.

And, you wanted to inhibit one of those steps. Any preference of where you'd like to inhibit the step? How about the first committed step of cholesterol synthesis? So, how about H, M, G, CO-A reductase inhibitors?

Well, it turns out that people found HMG CO-A reductase inhibitors.

Lovastatin, a fungal product inhibits HMG CO-A reductase.

And then, companies, Merck and then many other companies, developed all sorts of what are called statin drugs that inhibit that enzyme. I would venture to say that a large fraction of your parents take statin drugs to lower cholesterol.

And this is how it works. It inhibits the endogenous synthesis pathway at the step HMG CO-A reductase. And, in addition, if they do things like take bile acid binding resins, and there's some combinations of those things, and also controlled your dietary intake of cholesterol, and FH heterozygote can get a 60% reduction in LDL particle loss. That is mighty good.

That brings them down to normal. Yeah? Yeah? Yep. So we're not talking about totally removing it. We're talking about decreasing it.

We're bringing it back down to a normal level. Well, it turns out that the cells out there will up regulate their LDL receptors as well, and I haven't focused on that.

But they're taking care of themselves. It turns out, you have to worry about all these strategies. This is too clever by half because you're going to mess up things in the periphery.

But it turns out the peripheral cells take care of themselves.

They'll up regulate their LDL receptors enough to get things in.

And the big problem is that the liver's not doing its job.

But you have to do the clinical tests to see that that's the case.

This turns out to be a strategy for FH heterozygotes.

But I just said that many of your parents take these drugs.

Most of your parents aren't FH heterozygotes.

Perhaps none of your parents are FH heterozygotes.

That turned out to be one of the most remarkable outcomes of studying this rare genetic disease. Well, as it turned out, this strategy, which had been the understanding that had been developed from this exceedingly rare genetic disease, and the strategy that had been developed with an eye towards these FH heterozygotes turns out to also work in normal individuals with high cholesterol not because of a complete mutation in the LDL receptor, but because of, perhaps, other differences, genetic differences that are weaker. And in fact, tens of millions of people take these therapies that were developed for this very rare situation. This is a perfect example of where understanding a rare genetic disease points us to the basis for a physiological pathway, in particular, all these feedback loops that allow us to do something that helps everybody. So, this has become a major, major therapy.

Who were the only people who don't benefit from this particular therapy of tricking the body into up regulating the LDL receptors?

Homozygotes, because they don't have a gene to be up regulated.

They don't have a functional gene to be up regulated.

And so, homozygotes need something else. What do they need?

Gene therapy. The best idea that people have here since the liver regenerates tremendously would be able to take out some liver cells, add back genes for LDL receptors, and repopulate a liver with LDL receptor transgenic cells. And that would help them.

Anyway, this is meant to illustrate the power of rational therapy, that understanding things, can you imagine trying to do this by hit or miss? It was your design knowing the pieces that God is here, and this is basically what we're trying to do with all of medicine is get to the point where we can design things that really do work.

See you next time.