

**2000 and Beyond: Confronting the Microbe Menace**  
**Lecture 2 – The Microbe Strikes Back**  
**B. Bret Finlay, Ph.D.**

**1. Start of Lecture Two (00:16)**

From the Howard Hughes Medical Institute, the 1999 Holiday Lectures on Science. This year's lectures, "2000 and Beyond, Confronting the Microbe Menace," will be given by Dr. Donald Ganem, Howard Hughes Medical Institute investigator, and Dr. Brett Finlay, Howard Hughes Medical Institute international research scholar. Dr. Ganem, who will discuss how infectious agents are detected and how epidemics of infectious diseases arise and spread, is a professor of medicine and of microbiology at the University of California, San Francisco. Dr. Finlay, who will discuss bacterial diseases, antibiotic resistance, and the role of molecular biology in providing potential solutions, is a professor of biochemistry, molecular biology, microbiology, and immunology at the University of British Columbia in Vancouver. The second lecture is titled "The Microbes Strike Back." And now to introduce our program, the president of the Howard Hughes Medical Institute, Dr. Purnell Choppin.

**2. Introduction by HHMI President Dr. Purnell Choppin (01:37)**

Welcome back to the Howard Hughes Medical Institute for the second in the 1999 Holiday Lectures on Science. Our speaker for the second lecture is Brett Finlay of the University of British Columbia, Vancouver, Canada. Brett's research group has made important contributions to the molecular biology of bacterial pathogens, including those involved in illnesses such as typhoid, food poisoning, and stomach ulcers. Brett is the first Canadian scientist to speak at these holiday lectures, and I understand he's planning to show you some dazzling animations that have never been seen before. Before we run our introductory video about Brett, I need to say an early good-bye to you. In a few minutes, I am going to the airport to catch a plane to Stockholm. Guenter Blobel, one of our HHMI investigators at the Rockefeller University, won this year's Nobel Prize in medicine for his discoveries about how proteins find their way to the correct locations in cells through a kind of molecular zip code. I've known Guenter for many years, and I couldn't pass up the opportunity to be with him when he receives the Nobel Prize from the king of Sweden. I hope you will forgive me for leaving a bit early. Guenter is the seventh of HHMI investigators to win the Nobel Prize, five of whom are still active in research. One of the others, Tom Cech, delivered these holiday lectures 4 years ago. He will become the president of HHMI at the end of this month when I retire after serving 14 years at HHMI, 12 as president. It's been a wonderful time for me. I have had the privilege of working with outstanding scientists such as Don Ganem and Brett Finlay and playing a role in the support of their outstanding discoveries. However, few things have given me more pleasure than meeting students like you every year for these holiday lectures. You're curious, you ask great questions, and we need you to ensure the progress of medical science. I hope these lectures will inspire some of you to follow a career in science, either medical research or some other field. One day, maybe one of you will be leaving at this time to go to Stockholm to receive a Nobel Prize. So as I prepare to depart for Stockholm and for retirement, I want to thank everyone who worked so hard to make these holiday lectures a success, not just this year, but every year in the past. Of course, I extend a special thanks to Don and to Brett, and most of all, I want to thank you in the audience both here in the auditorium and via satellite. You are the reasons we have these lectures, and we want you to share the thrill of exploring the unknown and the satisfaction of making the world a better place. Now it's my pleasure to turn over the podium to Brett Finlay. He calls his lecture "The Microbes Strike Back." Once again, we will introduce him with a short video.

**3. Introductory interview with Dr. Brett Finlay (04:36)**

Science has always been an essential part of our family. Both parents are scientists. My mother looks after plants. She is a botanist. My dad is a zoologist. He looks at birds. And so all my life, I have been crawling around, looking at things, watching earthworms, watching pond samples, always asking why, trying to figure out biology. In our lab, we search out what we don't know. That's our mission, just to find something that we don't know and understand something a little bit better. We study how bacteria cause disease in people, so there is bacterial disease, there is viral disease. We focus on bacterial disease, and we try and figure out right down at the molecular level, even submolecular level, of how these organisms cause the havoc they do in people. Mainly the diseases that we study are those related to diarrhea. So we have a saying in the lab that diarrhea is our bread and butter, and this is what we focus on because we can manipulate the organisms we're dealing with. They are very common organisms--salmonella and E. coli--and they cause a lot of problems. The recent outbreaks with hamburger disease has caused much interest in what we do. I think that the failure of antibiotics has spurred us on to find more ways to treat these diseases. So it has a large impact on human health. One of the applications of our work is that we can come up with therapeutics. One therapeutic we're trying to push forward is the idea of coming up with an E. coli vaccine that would prevent hamburger disease. Instead of vaccinating people, we are trying a different trick. We are trying to vaccinate the carriers, that is, the cows. They don't get sick, but they have it. We're working quite hard right now on the development of that as a vaccine which would mean hamburger would be that much safer. Holiday lectures are a wonderful opportunity to encourage science students to learn a little bit about a particular area of science, to see how the people in the field work, to see how the experiments might go, to see what we're looking at, what are the issues. I think that snapshot of seeing that particular discipline of science goes a long ways. That's what I hope they get across in these lectures.

#### **4. Dr. Finlay describes objectives of this lecture (06:40)**

Good morning. I, too, would like to thank Dr. Choppin for his kind remarks and introduction. Now, Howard Hughes was a man of many interests. He is well-known as an aviator. However, another one of his major interests was diseases. Basically, he was concerned with diseases, especially the microbial diseases, the things he called germs. It is very appropriate that these lectures are on germs, microbes that cause disease. Also, I think it is very appropriate and thankful that he had the foresight to set up the HHMI, which has funded research in my laboratory for the last 8 years. So what are we going to do today? I want to take you into the microbial world. I want to show you what microbes look like. I want you to get an appreciation for it. I want to tell you how some of the ways we treat microbial diseases, past, present, and future. We are going to discuss why some treatments work, why some don't, why some are failing, and the incidence of infectious diseases. This is a fascinating world, but it's also an invisible world.

#### **5. Demonstration: Size analogy of bacteria (07:41)**

One thing that I have to convince you of is microbes are small. How small? Well, similar to Don, I'm going to do another analogy that here is our virus, bigger than the pill that Don had. This is our virus here. Small. This is our bacterium. The size of this. And 1 mammalian cell the size of this room. Big, big difference in size. They are so small, you cannot see them with the naked eye. Just to give you an example, if we have a ruler here divided in inches and centimeters and millimeters down here, the bacterium is 1/1,000 of a millimeter. So you take the smallest degradation of this ruler, you line up a thousand bacteria end to end. With your naked eye, on a good day, you can see between 1/10 and 1/100 of a millimeter, but you will never see a single organism. You can only see them on colonies. We have to rely on other methods to see them.

#### **6. How does one study bacteria? (08:42)**

So how do we study bacteria, and how do we divide them into groups? Just like the plant and animal kingdom, bacteria generally divide into 2 different sort of groups. This is based on a stain that colors them either pink or purple. It's called a Gram stain. This is characteristic of what their surfaces are, and that determines whether they are pink or purple. Actually, this purple juice that's up on stage, that's the same chemical you use to stain these things. What I did here is I took 2 pathogens we are going to talk about the next day that cause disease--the hamburger disease causing E. coli and listeria, which causes disease in children and older people--and Gram stain them. And you can see. You see pink and purple. The pink is E. coli. That's what we call Gram negative. The purple is listeria. That's Gram positive. This is how we then generally break down the bacterial pathogens into Gram negative and Gram positive.

## **7. Bacteria are everywhere (09:38)**

One thing is for certain. Bacteria are everywhere and numerous. Just because you can't see them doesn't mean they are not there. And I would argue we are actually more microbial than human. The number of microbes in and on your body outnumbers the number of human cells in and on your body by 10 to 1. You are more of a microbe than you are human in that sense. Of course, they are smaller. Another way to look at it is that 1 single gram of feces contains greater than the world's population, greater than 10 to the ninth organisms, and we have about 10 to the 14th organisms on our body. Where are those microbes? Well, they are generally on the body's surface. Skin, eyes, ears, nose, mouth, feet, wherever, arm pits – they are all over us. They are also sort of in us in the gastrointestinal tract, but that's not actually in us. That's actually a tube that goes through us. So, normally, blood, tissue, things like that that are really inside of us, microbes are not there. We go to great lengths to block the microbes from getting there. That's not a normal occurrence. That's usually sterile.

## **8. Role of bacteria in body (10:41)**

What do these things do? They play a major role in our lives. There are 3 fundamental roles that these things do for us. The first one is they aid in our digestion. Having all those microbes in your intestine digesting your food makes it easier for you because they do the dirty work. They break a lot of things down. The other thing is they actually produce things like vitamins that we can then utilize to assist our growth. So they are actually sort of a production factory inside us. From our point of view of this lecture, the other thing they do is they give a protective coating, sort of a blanket, to protect against pathogens. If a pathogen comes wandering in and it's going to hit a cell surface, if it's coated in microbes already, what's it going to do? It's going to try to get there, can't go, bounces off, and then gets cleared out of the system. So these normal flora actually provide a very protective barrier against incoming pathogens. Often you see secondary infections after someone takes antibiotics because they kill the normal flora as well. You're then more at risk for another infection.

## **9. Cheek cell demonstration (11:44)**

So let's have a look at microbes. Could I have a student volunteer that wants to come up here and help me? Ok. How about you? So we're going to go to the microscope now. Hi. I'm Brett. -Tom. -How do you do, Tom? Come on over here. Park yourself against the micro hunter's the main weapon, the microscope. This is how we have to do things. You're not going to see much. Don't worry yet. Let me tell you what I did. I did an experiment just to prove how these microbes are everywhere. I took a toothpick, scraped off the inside of my cheek, and put it on a slide and Gram stained it. We're going to see 2 things on here: one, the cells on my cheek, and the other one is maybe some of the normal flora that is on my mouth. Maybe you could look through here, and maybe we could project the microscope up here. Put your hand here. This is the focus knob. I want you to focus this. Try not to touch anything else except that. Gently do it, see if you can bring it into focus. Ooh, microbes! Of course! This is my cell. That's great, Tom. Thank you very much. For your hard work, you earned a U.B.C. T-shirt, a prized

possession. -Wonderful. -Thank you. So here we have a cell, my cell from my mouth, my nucleus, and my normal flora. Normal flora, you pick it up when you're born. You take it with you to your grave. It exists in you all the time. So it's everywhere. It's all over us. That's completely normal.

#### **10. Rate of bacterial reproduction (13:17)**

That was just a picture, again, showing the same thing. Then on the next slide is the concept of what do microbes do? They grow very, very fast. E. coli doubles in 20 minutes. What time do we double in? 20 years, 25 years? Obviously, they have a superior advantage in terms of their growth time. That gives them many, many properties to actually exploit their new niches and then live in various places. If we could have the first video. What we were going to show on this one is E. coli growing.

#### **11. Video Clip: Bacterial growth (13:49)**

Bacteria doubles, 2, goes to 4, goes to 8, goes to 16, goes to 32, and in 24 hours, we have 2 to the 72 microbes sitting there. They obviously have the capacity to divide very, very fast. They continue to grow. Then we're left with basically overrun with bacteria. So you see that, and you say how come we're here today? How come microbes just haven't taken over the world? This is in a lab situation where there's lots of nutrients. They can grow quite fast, and the ability to grow like that is really when nutrients are when there are lots of them. Inside the intestine, it's tough slugging. The E. coli only divides once every 24 hours in there. That's because it's battling out with all these other neighbors for the food, so it doesn't grow very fast. There is limiting factors. Unlimited growth takes unlimited energy. This world does not have that.

#### **12. Mortality rate and productivity loss from infection (14:44)**

The other thing is that most microbes are harmless. That is, they don't cause disease. But, as we all know, some do. So, infectious diseases, bacterial diseases, they kill a third of the world's population per year. Seventeen million people die of infectious agents. That leads heart disease, cancer, things like this. You say that's just off in the Third World. In North America, United States and Canada, infectious diseases are the third leading cause of death. Heart disease and cancer come up, but 20% in the U.S die of infectious diseases. Still, it's a major problem. Not only that, they are the leading cause of lost productivity. Your kid has an ear infection, you have to take him to the doctor. Measles, something like that, chicken pox, you have to stay home from work. All these diseases actually impact on productivity. Diarrhea might not kill you. You might wish you're dead, but it's miserable, but you got to stay home from work, so lost productivity. Dollarwise, \$120-130 billion per year in the U.S. alone, or 15% of our entire health budget, is spent on infectious diseases. So it's still a problem.

#### **13. List of the leading deadly bacteria (15:54)**

Let's have a look at some of the organisms that actually cause disease. These are the leaders. This is not a list of everything. This is just the numbers worldwide. This is the millions of deaths. Tuberculosis, caused by microbacterium tuberculosis, the most deadly bacterium. It kills greater than 3 million people per year. It's thought that 1/3 of the entire world carries TB. Not everyone is sick at that time, but as you get older and as conditions prevail, TB then may come up. Diarrheal diseases are many bacterial and viruses sort of lumped together. We will talk more in the second lecture, some of the diarrheal diseases we work on--3.1 million. Respiratory diseases such as pneumonia, a major problem. Then, again, you can see the other parasitic diseases. Not viral, not bacteria, but parasites such as malaria continue to cause a big problem. As we heard last lecture, HIV kills about a million people per year. So,

#### **14. Susceptibility to disease (16:51)**

some bacteria cause disease, but sometimes, not always, they pick on people that are sick, if they have weaker immune systems, for example. If they are young, if they are old, if they are starved. Those are the people more susceptible to bacterial diseases. And I like to think about the word "dis-ease"--maladies. It's a great word because it means you're not at ease. That means something is wrong in you. When a pathogen comes in, it's basically a battle between the pathogen and the host, and about 99.9% of the time, you win. The pathogen goes on its way, and you don't even know you have the disease. But it's that small fraction of the time when the pathogen does cause an infection, causes tissue damage, we then see that as disease.

### **15. Diseases kill more people than bullets do (17:34)**

Infectious diseases have really shaped our history. They have played a major role. In the Civil War, more people died of infections than they did of bullet wounds. The influenza pandemic that swept the world in 1918, it killed more people than died in entire World War I. For example, plague killed a quarter to a third of Europe, really earning it the Dark Ages name. Again and again, many armies, it's not the battles that defeat them, it's the dysentery in the troops. You read this throughout history. So throughout history, and throughout our future history, infectious diseases will play a major theme.

### **16. Bacteria cause many diseases (18:10)**

Bacteria cause many diseases of many different kinds. They cause all sorts. Some are rather surprising, shall we say, and others are rather expected. So what I'm going to do now is ask you to come up with, say, two or 3 examples of bacterial diseases coming from the audience. Someone suggest a bacterial disease. Put your hand up and yell loud. Strep throat. Yeah, that's a great one. You earned a candy cane, but I don't know how I will get it back there. Why don't you pass this back? It's a holiday lecture, right? Now I will get more answers. Someone else come up. Strep throat's a good one. E. coli. What kind of disease does E. coli cause? I don't know the name of it, but it causes some gastrointestinal diseases. It also causes other diseases-- meningitis and things like that. Here. I don't want to wing this back, so we'll just throw this back for you. One more. Pneumonia. Yes, pneumonia is a very good one. Yes, it causes a lot of problems. Thank you very much. So on the next slide are some examples of bacterial diseases. This is just a list I drew up. We could spend the rest of the lecture going through bacterial diseases. I don't want to do that. Some of them are obviously not life-threatening. If you have an important social engagement tonight, this may appear life-threatening, but it's not going to kill you. Teeth decay, dental cavities. This is actually the leading infectious disease worldwide. Over half the people in this world have dental cavities. It's caused by many pathogens – three hundred in the mouth, our normal flora. Maybe a handful cause disease. We can only grow 5 in the mouth. We still don't know a lot about what happens in the mouth, but a lot of people have this. All the ones, we can go through the list. You can start to recognize some of these. We'll hear about some of them in my next lecture. Tuberculosis we talked about-- the most deadly agent. We'll talk about typhoid fever next day.

### **17. Ulcers and other diseases caused by bacteria (20:05)**

Let's talk about ulcers. If you want to ask your parents or grandparents, everyone will say ulcers are caused by stress. You work too hard, you get ulcers. This was the dogma. You gave antacid and things like that to fix it. In 1982, this Australian named Barry Marshall, he kept seeing – he was a gastrointestinal doctor – he kept seeing bacteria in the stomach. He said, "Maybe those things are causing ulcers." Everyone said, "You're crazy. Ulcers are not bacterial." Then he did the ultimate experiment. He swallowed these bacteria. And what happened? He gave himself ulcers. Then he did the real neat experiment. He took antibiotics, and then he fixed it. The ulcers went away. It was an uphill battle. He had to fight a lot of dogma, but the bottom line is ulcers are caused by a bacteria, something

called helicobacter pylori. It's thought that over half the world's population has this organism, and now, of course, you use antibiotics to treat it. Now, this is a neat pathogen because not only does it cause ulcers, it also causes stomach cancers. This bacterium is classed as a carcinogen in the same class as a cigarette. So here we have a bacteria that's actually a carcinogen. Now, viruses are known to cause cancers, but this is the first time a bacterium has. The other thing we are seeing is there's still diseases that we don't know what causes them. There will be some diseases in the future that are caused by bacteria we didn't suspect. For example, atherosclerosis, which is hardening of the arteries. Everyone says that's due to diet. That data is now starting to mount that it actually might be of bacterial origin. Inflammatory bowel disease is probably of bacterial origin. We haven't proven it yet, but the hunt's on for these things. My guess is we'll have many more surprises. So,

### **18. How do bacteria spread? (21:45)**

how does the pathogen get around? Well, it has to cruise around the world, and, really, the ways to enter a body are orally. You swallow it. Fecal oral transmission is a very common one in the contaminated water and food, or it can be airborne. One thing's for sure. We live in a very, very small world. So,

### **19. Demonstration: Air transmission of bacteria (22:07)**

here we have a story I'm going to tell you. This is 1994. A woman with fulminant multidrug-resistant tuberculosis flew from Chicago to Honolulu-- 9-hour plane flight. On that plane, she did what people with TB do. They cough and hack and things like this. And this is the seating plan of the plane. What we have done, if you imagine that in this auditorium we have the seating plan of this plane going up here, what I would like you to do is all take that thing that pulls out that you write on and pull it up. Some of you will have colors on it. Including you at the back. Make sure you pull it up. There should be--are there some colors coming up? What colors do you have? Blue. There should be one right up in the back that's-- What color do you have? White. -I'm sorry. What? -White. Perfect. Congratulations. You have multidrug-resistant tuberculosis. "Oh, no!" she says. Can you stand up, please? People with TB can stand up. You have tuberculosis. Can you be a tuberculosis patient? Remember, you want to cough up blood and sputum and all those lovely things. Can you do that for me, please? No, we got to infect the whole planeload. One more time. That's good. OK. We're getting there. She sat on this plane and coughed. OK. Now can the people with the blue coding all stand up? Stay standing up, please. These are the people that then got it. They were then exposed to this. They actually seroconverted to this. OK. You can see how it spreads. It doesn't necessarily mean the person beside her gets it, but the odds are within the first 2 rows, you stood a better chance of getting it. You can be seated. And for my lovely tuberculosis patient – in real life, unfortunately, this person died of multidrug-resistant TB. But this is not real life, so you get a U.B.C. T-shirt. Can you pass that back, please? Thanks for doing that. All right.

### **20. How do we fight bacteria? (24:15)**

How do we combat infectious diseases? We have 2 main tools. One are antibiotics. These kill organisms. They don't kill us. They kill microorganisms alone, and you use these diseases once you have an infection. The other main tool we have are vaccines, and we get vaccinated against them. They do that by stimulating our immune system to go and fight these microbes off when we encounter them. When vaccines work, they work great. They are used to prevent the disease rather than to treat it. That's a fundamental difference in these treatments. So what I'm going to do is go through both of these and discuss them a little more.

### **21. Antibiotics: Penicillin structure (24:51)**

Antibiotics changed the way our society works. They were a major, major discovery. I would like all of you to go home and talk to your grandparents what it was like to live in the pre-antibiotic era. Very different world. Diseases such as gonorrhea, relatively simple disease to treat, you can now treat it. Before, you couldn't. That just changed the way you thought about diseases. This was a huge discovery. So, antibiotics, what are they? They are small chemicals, compounds, that kill microbes. The sulfonamides were discovered in the 1930s. Penicillin came into light in 1940, and it was used just in time for the end of World War II to really change infections. This is the structure of penicillin. This is something called a beta-lactam ring. That's a key part. You'll sometimes hear things called beta-lactams. That's because they have this structure.

## **22. Origin of antibiotics (25:42)**

So, where do you think these things came from? Does anyone know where antibiotics came from? I'm sorry. I have to ask someone to put their hand up and take a stab at it. OK, way at the back. Moldy bread. Got the mold part right. So, mold. OK. Knowing that, how come moldy bread has antibiotics-- mold-- in this case? You have no idea. Anyone else want to help her? Maybe it was discovered by chance. The discovery was by chance. I want to talk about where these antibiotics came from. They were pre-existing. Mold has them. Any ideas? Maybe the bread was trying to fight off the fungus by expressing antibodies. Close. The soil is a war zone. Basically, the bacteria are fighting and everything. If you made antibiotics, you would kill off your competition. That's why mold and other organisms make antibiotics. Then can kill it. So antibiotics actually came from pre-existing soil organisms that beat on their neighbors to decrease the competition.

## **23. Antibiotic modes of action (26:51)**

Antibiotics work by blocking essential bacterial things, pathways. These things are pathways that are different or somehow different than our pathways. You don't want them to be toxic to us. These are some of the steps that they can interfere with in the bacteria. Penicillin works by putting holes in the cell wall. They block DNA replication, RNA transcription, protein synthesis, or they can poke holes in it.

## **24. Culture plate showing antibiotics' effects (27:20)**

On the next slide, we have *E. coli* plated out. In this case, what we did is put different antibiotics on these different disks, and you can see that when – this is the *E. coli* growing here, around the circle here, there's a zone of clearing, antibiotics diffused out and killed these bacteria here. That means they are susceptible to it and it kills them. They get a nice killing of the bacteria. What we are going to do next is go to a video that will show *E. coli* become lased by the penicillin. So go ahead and roll the video.

## **25. Video clip: Penicillin killing *E. coli* (27:54)**

We have *E. coli* growing here. It's living, you can see it is starting to grow. Then we are going to add penicillin. You will see the bacteria – they are going to pop. There wasn't any microphone on this, so we couldn't catch the pop sound. What you'll see is they will suddenly start to go clear. There goes another one. It's poking holes in the cell wall. Boom! Bacteria is dead. So that's what's killing them. Works great. OK, and we are going to go back to the next slide.

## **26. Antibiotics increased life expectancy by eight years (28:28)**

So antibiotics are the absolute wonder drug. And these are – they were so wonderful that it really changed the way we thought about things. And they work against bacteria, not viruses. Just to tell you how wonderful they work, they increase the average life span of a person by 8 years. That may not

sound like much, but all the medicine that we've learned since 1970s increased our life span by 2 years. Huge, huge increase in our life span. In typical people fashion, we got very confident, and we said, and the U.S. Surgeon General in 1969 said, It's done. It is time to close the book on infectious disease. We won. Never ever doubt nature. It is an unfortunate statement that's come back to haunt the whole area because – not yet. Well, what happened?

### **27. Bacteria can develop antibiotic resistance (29:20)**

We got antibiotic resistance. Here you can see the antibiotic put on this disk cannot kill this organism. It is resistant to it. So now the bacteria can fight off this, and they have ways to then actually fight this off. So the bacteria acquired this resistance to antibiotics. They went around, and they actually picked up genes that encode the resistance. And one of the ways we got the resistance is because these bacteria can divide very fast, and this then puts a selective pressure for them to be resistant. This is, again, an example of society impacting biology, in this case, disease.

### **28. How does antibiotic resistance develop? (29:58)**

There's 3 real major reasons why we have antibiotic resistance. The first one, which is not really a problem anymore, at least in developed countries, was physicians using the wrong antibiotics at the wrong time. Person has a viral infection, they give them antibiotics. Antibiotics don't work against viruses. It's not going to work. Or giving them, say, a fourth-generation cephalosporin when basic penicillin would do – sort of inappropriate antibiotics. I would argue that this, at least in our society, is not that big of a problem. But also driving this are the patients. You have a child with an ear infection, go to the doctor. The doctor says, "It is viral. "There is nothing I can do." You say, "No, give me an antibiotic. "I want an antibiotic. "If you don't give me one, I can go to another doctor." There is patient demand there. The other thing is patient abuse. When you take a tube of antibiotics, it says please take until complete. The reason they say that is if you only take it halfway, you feel better, you throw it out, not all the bacteria is necessarily dead. You're selecting for resistance. Again and again, that's how we see resistance arise. A tuberculosis patient has to take drugs 6 months to a year. Try and get a homeless person in New York to take a pill every day for a year. Compliance is a big problem. That's why we see resistance. The other thing is agriculture. Over half of all antibiotics are used in agriculture. This is bad news. Often we see a farmer presenting at a hospital with an infection that's resistant to antibiotics and resistant to exactly the same thing, the same antibiotics, given to his livestock or fish or pigs or chickens or whatever. We see this over and over again, so we can't use it in agriculture.

### **29. Superbugs (31:34)**

I want to talk about the super bugs, antibiotic resistances. And if I could get you to take one of these and pass it around randomly. I will go way over to the other side here. Take a piece of paper and pass them along and see what we're doing in a moment. "Superbugs," press has coined them. What is a superbug? That just means it's resistant to things. What I want you to do, those of you taking paper, when you have 2 colors, the first person to get 2 colors, please tell me. Please hold up your hand or wave or something like that. So, the superbugs we know is something called methicillin-resistant staph aureus. This is staphylococcus aureus that is resistant to every antibiotic except vancomycin. We have one drug to treat it. So then there is the other disease, other bacteria called vancomycin-resistant enterococcus. This thing is resistant to everything. The reason I don't think it's a real superbug is because it really only infects very sick people. Staph aureus, on the other hand, is resistant – is a much more serious pathogen. So do we have 2 yet? OK, no? Keep passing. You can see resistance is going up, and again and again. The scary thing we're worried about is what happens when real pathogens like staph aureus are resistant to everything, and that's what we will see happening. OK, so how do the microbes do this? Have we got 2? Oh, you don't want to admit you have 2. Freeze frame right over there. Ok. So, congratulations. So what

we have done is shown you the idea that you can move the resistance around, and what has ended up with her is she now has staph aureus that has acquired the resistance from the vancomycin resistance in the enterococcus. You now are resistant to everything. That means that you are something called a vancomycin intermediate staph aureus. They call these things "visa." That's a scary bug. That's resistant to everything. So seeing as you are so scary, I have a candy cane for you. Want to pass that back to her? So this is – this problem first occurred in 1996, and these are what I think are the real superbugs because they are resistant to everything. We just can't treat them. And we go back to the pre-antibiotic era.

### **30. Bacteria can share antibiotic resistance (33:47)**

So where do these things come from? Well, if you want something, where do you look? You look on the web, right? Well, at least I presume you do. That's where I look for things now. And microbes have their own internet. They have the genetic internet. What they can do is go and get antibiotic-resistant genes from their neighbors and download them, just like you do to a program or something. Because it's in the soil, there are resistant genes kicking around. The bacteria can go and get them and pass them around and share them and keep them – put them in their back pockets in case they hit antibiotics. So they obtain these resistances. There are sort of 3 real ways you can do it: something called conjugation, which DNA goes from one bacteria directly to another. So you just transfer it like you directly download a file. You could something called transduction--there's a virus. That's they give a floppy to a person that passes it along, and then you get it. Or you can go and pick up the DNA – something called transformation. You grab the DNA and yank it in your cell, like going to the software store and buying it directly. What will come next is a video, and what this video is going to show is an example of conjugation. OK, go ahead, roll the video.

### **31. Animation: Conjugation (34:54)**

We are going to have 2 bacteria, one here, this is resistant. Here's an antibiotic resistant gene. It's going to then make something called the pilus and transfer the gene over. Boom. It encodes this thing. Now we have an antibiotic-resistant gene in here. And this organ now has the resistance of the antibiotic. Again and again, bacteria do this. Again and again, we see this happening in our bodies.

### **32. Development cost of antibiotics (35:18)**

OK, so what do we do with these antibiotics? And just to tell you how limited we are, here is the current scenario facing someone that has a new antibiotic in the pharmaceutical agency. They sunk \$200 million at least into getting this thing approved and proving it safe and proving it worked. It's taken 10 years since the initial eureka at the bench side to get this thing out. That's normal for a drug. So you've invested a lot in it. What we currently see, you've got about a year of wide usage, and then you see resistance cropping up. Within about 2 years, basically, you can't use it anymore. So to recover your costs and effort, it's huge, and this is one thing we're facing. So,

### **33. How should we use antibiotics? (36:03)**

what are we going to do? What do we do? Say I'm a pharmaceutical company, we've got this new drug. It works great. What do I do with it? Anyone have any suggestions? Use, like, 5 at once? Use, like, 5 at once. That wasn't quite the answer I was looking for. You could think about that. The idea is kill it and kill it dead. What I was saying, let's say we have one that can use one antibiotic, but let's say it's not resistant to number 3. That way if you use 5, it's not going to be resistant to all of them. Right. Certainly. OK, what else do we do? Yeah? Hold it in reserve until we actually need it? Hold it in reserve? Yeah. That's a good idea. Or just use it appropriately, for certain diseases. Last one. Market it in as many places as you can. Sell the heck out of it, make lots of money and move on. Are you a scientist or an

MBA? I would argue, treasure it. Antibiotics are a golden gift. They work. But we can't abuse them. You can't feed them to livestock and humans at the same time. Don't use it in agriculture. Do it worldwide. For example, in Asia, antibiotic resistance is a horrible problem because you can get any antibiotic whenever you want it. So people take chloramphenicol a day to keep the bugs away. I mean, it's very common. So treasure it, use it wisely, and, of course, make new drugs. The next lecture, I will talk about some of the ideas we have for that.

### **34. Vaccines: Basic facts (37:25)**

Ok, let's turn a little bit to vaccines. Vaccines are wonderful when they work. They have major impact on disease. And vaccines are unlike antibiotics. They don't kill the pathogen, and so you don't select for the resistance, like you do with antibiotics. And vaccine resistance is not nearly as common as antibiotic resistance, so they work. Because you're just sort of saying no, you can't go there. You can live over here, but you can't live over here. The way vaccines work is they basically take all the body's normal immune system so that it says, "be prepared. You're going to see a pathogen that looks like this, and when you see that pathogen, you then go into action and really neutralize it. So you're warming the body's immune system up. You may never see that pathogen in your entire life. You're vaccinated for it. You just don't see it. So that's an important point. So what do vaccines consist of? They are actually very simple. They are either a piece of a pathogen like, say, a molecule on the surface, or they are a crippled pathogen that can get into you, but it can't cause disease. This is something called the live attenuated strain. And vaccines, that's what they are. So when you put these things into the body, the body recognizes either that piece or that crippled pathogen, and then it has this immune response, so the next time it sees the real McCoy, it can then go after it, and then your own immune system will take it out. Vaccines work. They are cheap. Once you make these things, you can make them in large quantities. They are relatively easy to administer, and the neat thing is they sort of provide a blanket immunity. If 90% of you are vaccinated and a pathogen comes wandering in through the air ducts and lands on one of you, the chances are it's going to land on someone that is vaccinated, and then it's going to stop. If per chance it hits the 10% that didn't have it, the chance of it jumping to the next person that has also been vaccinated is very great, so you block its transmission. This is a wonderful safety feature of vaccines because we basically take out the whole human population, and it says you can't live in there, and you often break the chains of command or chains of infection to it. Here is a question.

### **35. Should everyone be vaccinated? (39:31)**

So if we have a great vaccine, let's say it's got a 1 in a million chance of causing seizures, yet the death rate to unvaccinated kids is one in 300, what do we do as a society? What happens if you say I don't want my kid vaccinated. Got a 1 in a million chance of getting a seizure. I should add that the chance of a normal seizure is still 1 in a million. But this has been an issue, and this is an issue that was exactly discussed with whooping cough vaccine. That's what we were faced with. And it's not for scientists to answer. I would argue this is an ethical question, and it's for society to answer. But I also argue that for the good of society, we should vaccinate. It's unfortunate, but the chance of dying of the disease is much greater than having a side effect of the vaccine.

### **36. List of available vaccines in 1959, 1982, and 1999 (40:12)**

So here we have a list of vaccines. This is the year I was born. This is what was vaccinated back then. This is about the year you were born. These are the concepts of the vaccines you could have. You can see we have added several to the list. I had these diseases—measles, mumps. I remember mumps. It was an awful disease. You're like this, sort of like a squirrel. I actually remember Sunday evening dinner, we're having roast beef, my favorite dinner. I couldn't eat it, my throat was so sore, so my mother did me a big favor. She put it in the blender, made this brown slurry, and then I got to drink it. It didn't quite cut

it. It wasn't the same thing. You guys won't get mumps. You're vaccinated to it. Today the list grows longer. And look at this. We can now start to cross off some. Polio, I'm sure in your lifetime, will also be crossed off this list. Great diseases. Diseases, that, say for example, chicken pox. How many of you have had chicken pox? My guess is you have all had chicken pox. When I ask that question to your kids, the odds are no hands will go up because chicken pox, we now have a good vaccine to it. So it continues, and we continue to add to this list.

### **37. How do we develop a vaccine? (41:17)**

Well, how do we develop a vaccine? It's really hard to sit down and say, how do I rationally do a vaccine? You have to understand the pathogen, you have to understand the immunology, and you have to basically understand what's going to go on. And it's by chance. Some work, some don't. The other thing is some pathogens change their surfaces. Tomorrow when you show up, you may have a different colored sweater on. You may look different to me, and I might not recognize it. Just like the bugs do, they change their surfaces. I can tell you a brief scenario. The U.S. Army wanted to develop a vaccine to gonorrhea. It was a problem. So they took this one surface molecule and gave it to rabbits. Rabbits made nice antibodies to it. But gonorrhea only infects people, so they can't test it. So how do you test it? So in the army, there's U.S. Army volunteers, right? That's where the term comes from. They vaccinated these people. Then how do you challenge for this disease? They threw them all in a boat, sent them to the Philippines, where there is a high incidence of gonorrhea, and let nature take its course. The vaccine didn't work. These people still got gonorrhea. They had a penicillin supply, so they treated it. Why didn't it work? A rabbit's immune system is very different from a human, and gonorrhea is able to change its surface, and that molecule they're vaccinating against is constantly changing. So science, you got to understand the pathogen, you got to understand the disease, and you got to then figure it out to make a successful vaccine. And there has been lots of successes recently. For example, influenza causes kids' meningitis no longer. It vaccinated against it. It's an abnormality when you see with this disease. And so they are working.

### **38. Genomics of bacteria (42:52)**

So we have one final ace up our sleeve, and this is an area that is going to impact significantly under science. It's something called genomics. This is the future. This is where we're going to really start to get some neat stuff going—something called pathogenomics, which means genomics of bacteria. And very soon we'll have the entire DNA sequence of all the major microbial pathogens. There's about 20 done now. Well, if this is a phone book, if each of these letters in the phone book was an A, C, G, or T, how big would a microbial pathogen genome be? About 4 million characters, which is the first 100 pages of a phone book. That much information in an organism. That's what each of these genomes has demonstrated. So a lot of information. The scary part is, when we see *E. coli*, we have been studying this for 50 years plus, we only knew what 60% of the genes were doing. 40% we had never seen before. We didn't have a clue. So it tells you where we are in our knowledge. The next few years will be spectacular in what we learn. We can knock out every gene and ask which one does *E. coli* need to live or die? Essential genes, great targets for antibiotics and even vaccines. And what you can do is you can compare pathogens to nonpathogens and say where are the virulence factors, because if they are in this pathogenic *E. coli* not in lab-strain *E. coli*, we say, "Aha!" then we can go after those things. So what you can do—this doesn't show up very well, but each of these dots represent one gene, and there's 4,000 of these here. These are the 4,000 genes of the tuberculosis vaccine. This is a group in Montreal and Stanford that did this. Basically each of these little dots is one gene from the tuberculosis vaccine, this thing called BCG. And they can then study each of these genes, say an infection, which one goes on, which one goes off. They can compare 2 related strains and ask at a genetic level, how similar or different are they? They can also say is this TB, or is this something else? These are very useful tools to

have.

### **39. Bioinformatics and the Human Genome Project (44:46)**

So this is something called bioinformatics. How do you deal with all those 4 billion plus A, C, Gs, and Ts? You use computers. If you like biology and you like computers, this is the career for you. This is a wonderfully exciting area. We need computers to just handle all this information. And, for example, in our lab, we're subtracting entire genomes of a pathogen from a nonpathogen using these computers. Wonderful technology. Five years ago if you said I would do this, I would have said you're crazy, but the computers play a key role for that. So there is a big area. The human genome, last week, major milestone, the first DNA sequence of the entire human chromosome, chromosome 22. Within the next 2 years, we're going to have the entire human genome, 4 billion base pairs. We take this phone book, stack them up, we would get a stack 3 meters, 10-foot high. That is how much information is in one mammalian cell-- huge amount of information. You need computers to deal with it, but it's all there. So...this is big, this is really big. And then we can also use this to determine how, for example, the host responds to an infection, what genes in the host are actually involved. So this is a student in my lab, Terry Rosenberg, who put 600 macrophage genes out and infected them with salmonella. You can see some host genes come up. If we go after the host genes, we now understand how the host responds to infection as well as the bacteria. So...

### **40. Who will win—humans or bacteria? (46:16)**

what's going to happen? And more importantly for this audience, who is going to win? Are we going to win or the microbes going to win? Well, we are after a stalemate. There is no way we're going to live in a sterile world. You cannot plot that. So what we want to do is treat certain disease so we don't have to deal with them, but I would argue that we will never get rid of all infectious diseases, and there will be always new ones coming up. We just constantly have to be on our toes. Science is cruising. These are very exciting times right now. You guys are really lucky, because what you will see in the next few years is absolutely amazing science. We will get genomes, we're going to get transgenic animals, we're going to get neat models to study. All these tools will allow us to fight these infections really significantly. So being a microbiologist, I always have to be self-sufficing and plug microbiology. It will be a great time. We have not closed the book on infectious diseases. There is a lot more excitement to come. So my time is up. What we're going to do

### **41. Student question: Why don't we have an AIDS vaccine? (47:14)**

now is go to questions. I can take a question from you people. So we will go way to the very back there. Before I—make sure you speak loudly so we can all hear you. Go ahead. I'm from Thomas Edison High School. Is it true that we don't have a vaccine for AIDS because of the antigenic variations in the code? That's a good question. Why don't we have a vaccine to AIDS, and is it due to the antigenic variation? Yes, that's true, AIDS is a changing virus, and it's constantly changing, and it's been very difficult to find something that's always the same that we can then fight it with. We have been successful with AIDS in creating drugs that block various viral processes. There is this thing called the triple therapy. In developed countries, that's really decreased the amount of serious AIDS infections. So, yeah, if the vaccine doesn't work, there are other ways to it, but you're absolutely right.

### **42. Student question: Do disinfectants affect resistance? (48:05)**

OK, we're going to go one more. How about the guy in the black shirt right there? Speak up, please. How do antiseptics and disinfectants contribute to the resistance? How do antiseptics and disinfectants contribute to the resistance? Well, disinfectants usually kill bacteria. Usually it's chemicals like phenol.

That's what's in these things. That's a chemical thing. So unless it's a specific antibiotic, it doesn't really contribute to it. But they actually are not—the idea of wiping your counter down with an antiseptic, I have done the experiment. You then go and swab it, you'll grow bacteria just fine. You're not killing them. You're killing some of them. In Japan, you put your visa card in a teller machine, and it autoclaves it so there is no more bacteria. But you pull it out, you grab it with your fingers, right? What's on your fingers? Thousands of millions of microbes. So we can't live in a sterile world. But that I don't think is contributing to it.

**43. Student question: Can we use viruses to kill bacteria? (48:54)**

I guess now we will take a question from Penn State. Go ahead, Penn State, please. My name is Burt Neely, and I'm a senior at West Perry High School from Ellisburg, Pennsylvania. I was wondering, are bacteria susceptible to viral infections, and if so, might viruses be a potential way to control bacterial infections? The question is, couldn't we use a virus that kills bacteria to then kill the bacteria? It's been tried. The Russians actually worked on this quite a while, and they actually were moderately successful at it. For some reason, it really hasn't been adopted as a therapeutic. One of the problems is viruses are proteins, so when you add a virus to a person, you're then basically vaccinating the person against that. It might work once, but it won't work again. So it just hasn't been adopted yet, and I think generally there have been some problems with it, but it's a great idea, and I'm sure several people continue to work on it. Thank you.

**44. Student question: What bacterial disease causes blindness? (49:45)**

OK, we'll go back to the house now. Go ahead. ...Examples of bacteria diseases. I was somewhat surprised to see blindness, and I was wondering what its bacterial origins are. The question is they saw blindness on our list of diseases. There is organism called chlamydia trachomatis. This is the leading cause of blindness worldwide. You get it through the eye. Causes eye infection, causes inflammation of the eyes, scarring of the eye, and it is the leading cause. It's mainly in developing countries, but it's a huge problem worldwide. It is also the same one that causes sexually transmitted diseases, and you see that in sexually transmitted diseases in our society—same organism. Yeah.

**45. Student question: How do we deal with biological warfare? (50:26)**

OK, now we are going to go to Miami, I understand. We have a question from Miami. Go ahead, please, Miami. Hi. My name is Diego from Miami Northwestern High School. With anthrax as an example, what is the scientific community and the government doing to prevent people from using biological warfare? Ah, biological warfare and bioterrorism. This is an issue obviously, and what do we do? You could argue that if it is so easy to grow. There are several ways we are trying to treat this. Biological warfare is certainly not new. It's been used over the centuries. They were catapulting plague victims into besieged cities. They were spreading smallpox on blankets to the Indians. Biological warfare is used. What we are doing and many people is A., we have to be able to detect what it is relatively fast; B., we have to then know how to treat it. There are many, many initiatives going now to try to come up with ways of treating it. It is certainly—we don't have all the answers, but I would argue we are well on the way. It is an issue we have to be aware of.

**46. Student question: How does flesh-eating disease work? (51:29)**

OK, I understand we are going to go back to the house now. Actually, right here. Speak up, please. On your list of bacteria, I saw flesh-eating disease. How does that work? How does flesh-eating disease work? Yeah, boy, when the press get a name, they really get it. This is the same organism that causes strep throat. It's just bacteria at the wrong place at the wrong time. It's an accidental disease. One in a

million chance. If it gets in a cut or cut yourself shaving or whatever, bacteria gets in the tissue and makes enzymes that chew up the tissue. It's a very rapid disease. They basically just turn the whole tissue to jelly. They degrade it. Antibiotics don't work fast enough because in 2 hours, you will go down the whole leg. It takes overnight for antibiotic to work, so radical surgery, they just chew away the tissue as it progresses is our current treatment. Very serious disease and has a one in 2 chance of killing you, but it is very rare. One in a million, there are more diseases I would be concerned than that one.

**47. Student question: How do resistances initially develop? (52:24)**

How about here in the blue? My name is Leonard Lazar from the Jewish Day School. I was wondering, how do bacteria initially develop resistance to antibiotics? Well, they get the resistance by getting gene code resistance. That's what the video showed us. They acquire resistant genes. They get the DNA that says you make resistant into the bacteria. Once it is in there then it codes the resistance. They share these genes and mix and match them and sort of tuck in their back pocket. If they have the—if they hit the antibiotics, they have the resistant gene. So it's mobile genetic elements is what we call it. The genes are in nature already. Well, most of them are. Most antibiotics that we made in the last 30 years are theme and variations, so you can imagine new resistance genes are theme and variations of the old resistance genes. We see that a bit. Any time you put a selective pressure on an organism that can divide this fast, these kind of numbers, if you have 10 of the 9 organisms, a billion organisms, if one is resistant, it will come out of that pool immediately, and it will be resistant. Just by putting the selection pressure on, we enforce the development of the resistance to that. Bacteria are always mutating, so they are trying new things to see what works. When it works, they grab on to it.

**48. Student question: How do we treat different areas of the body? (53:42)**

We are going to another question from Penn State. Go ahead, please. Good morning. My name is Nick. I'm a senior at Hershey High School. My question is, harder to treat a bacterial infection in one area of the body than another? I think the question was is there a certain area of the body that bacteria likes to go to versus another? We have 9 openings in our body. Bacteria likes those places. They're nice mucus membranes They live in the skin. Skin is tough to infect. It is like a chunk of Gore-Tex or something. It is tough to get through. Bacteria can infect anywhere. They love it inside cells, inside tissue that's warm, that has lots of nutrients. That's microbial nirvana. Assuming you can get around all of the defense mechanisms... So pretty well. We are biological mass, and they like that.

**49. Student question: Can we use antibiotics years later? (54:26)**

OK, thank you. Now I understand we will go to Miami now. OK. I'm Crystal. I was wondering, if a physician stops prescribing a specific drug, can it be used years later? That's a really good question. Can an antibiotic be used later after we put penicillin aside for 50 years? Yes, it can but you will never get rid of the resistant genes. They're always there. As soon as you put selective pressure, it will come roaring back again. So what we've learned is that you can use it in certain cases. If we come with a widespread usage again, you will have resistance much faster because it is on the genetic internet.

**50. Student question: Can we modify antibiotics to overcome resistance? (55:14)**

Then it spreads really fast. It is a good idea but I don't think in terms of therapies unless it is used correctly. OK. We have a quick house question. Last one here. You were talking about the mutations. That's the way that the bacteria adapts in order to fight the antibiotics. It is a long shot. But couldn't our antibiotics be genetically enhanced to adapt to their adaptations? Yeah. This is the whole evolution and antibodies coming back and stuff. We haven't developed antibodies that do that. That's very difficult to do. I think we stand a better chance of understanding what the new unknown genes are, brand-new

target. Let's go after that, and then see if it works. At this time unfortunately we are out of time. I would like to thank you all very much. I will turn the floor over to Joe. Thank you.

**51. Closing remarks by HHMI Vice President Dr. Joseph Perpich (56:05)**

I'm Joseph Perpich, vice president for grants and special programs of the Howard Hughes Medical Institute. I want to thank both Don and Brett for their superb presentations today. We learned how scientists, like detectives, read the clues and follow the trail to the suspected organism of an infectious disease. Once they have got it in their clutches, then they interrogate the suspect, through their research in the hopes to find effective treatments or prevention. We have seen again this morning the growing synergy between epidemiology in the field and laboratory science. The mists are beginning to clear on how these organisms you heard about today do their business on human populations. In the not too distant future, today's treatment failures will become tomorrow's therapeutic triumphs. We look forward to having you all back again tomorrow morning when Brett Finlay and Don Ganem give their 2 final lectures. Brett will describe his research on how bacteria interact with our cells and cause disease. Don will tell us about the forces that create and shape epidemic infections. So we will see you again soon tomorrow morning at 10:00 a.m. as we begin the second day of the 1999 Holiday Lectures on Science. Thanks very much for joining us.