

2000 and Beyond: Confronting the Microbe Menace
Lecture 4 – Emerging Infections: How Epidemics Arise
Donald E. Ganem, M.D.

1. Start of Lecture Four (0:16)

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 fourth lecture is titled... And now, to introduce our program, the vice president for grants and special programs of the Howard Hughes Medical Institute, Dr. Joseph Perpich.

2. Introduction by HHMI Vice President Dr. Joseph Perpich (1:41)

Welcome back to the Howard Hughes Medical Institute for the fourth and final Holiday Lectures on Science. Our speaker is Dr. Donald Ganem. This morning he'll explain how genetic changes in microbes or natural or man-made changes in the environment can result in the rise of a new epidemic. We'll learn how microbes, like quick-change artists, can adjust their genetic expression to survive in response to environmental changes. Microbes can be our friends as well as our body's foes. Hundreds coexist happily within us, vital to our body's functions. They prevent many bad actors from the microbial and viral world from establishing an ecological niche within us. Dr. Ganem will conclude this lecture series by discussing opportunities for new insights into how the evolution of our world drives the evolution of the microbe world and vice versa. We will once again have a brief video in which Dr. Ganem describes his work in the different worlds of medicine and science. Whether in the world of science or medicine, Dr. Ganem loves to teach. Through his teaching, his students learn, as Dr. Seuss tells us, that "with brains in our head and feet in our shoes, we can steer ourselves any direction we choose." His life and work in science and medicine show that we can move mountains as Dr. Seuss urges us to do. The trailblazing research of Doctors Finlay and Ganem has opened up new vistas for insights in the role of microbes in human disease and human health. An awesome journey lies ahead for future generations of scientists drawn from students like you here today. Listen to Dr. Ganem's lecture this morning and think about his work and that of Dr. Finlay. Fortune has smiled on them. They have pursued their work with the perseverance of a Mount Everest climber and with the heart of a poet. Their work constitutes the very first chapters in the book of molecular medicine for the 21st century. Who among you in the audience today will author a chapter or two in this 21st century book of life? To do so, believe in yourself and keep in your heart these final words of Dr. Seuss: "And will you succeed? "Yes, you will, indeed, 98 and 3/4% guaranteed." And now we turn to the fourth lecture in the series to be given by Dr. Ganem: "Emerging Infections: How Epidemics Arise."

3. Interview Dr. Donald Ganem: What I like about being a scientist (4:16)

I like everything about my job. I just like everything about it. First of all, I like the fact that we have a tremendous amount of freedom in this line of work—freedom to think about the problem in any way that we want, freedom to organize our time, our effort, in researching the problem, to be the master of your own intellectual approach to a problem. That's just terrifically fun. One of the things that I find interesting about my own career is that because I have one foot in the world of science and one foot in the world of medicine, I've come to appreciate how different those worlds are, and you have to learn

how to operate with much less precision in the world of medicine. You have to be able to accept answers that aren't nearly as precise as the ones you are seeking in the world of science. When you go into the world of medicine, it's a world of real human problems, huge human problems—people who have terrible illnesses or other life problems that are very massive. And compared to your problems in science, you realize that no matter how hung up you get on a particular question, how insoluble a particular question is, what you learn when you go to the wards is that those things that yesterday looked like such big problems to you are really just technical difficulties compared to the real-life problems of ill people and how massive those problems are, and I find that very helpful. It helps me get my equilibrium sometimes when I have gotten too immersed in an intellectual problem that I'm having difficulty solving. I think if a high-school kid is interested in science, they ought to follow their natural inclinations about it and don't be afraid. Don't be afraid. This is a very accessible career. There's a lot of things to do here, and there are a lot of paths. This career can take you to a lot of interesting places, not just geographical places, but intellectual places. I can guarantee you that you will have fun along the way. If you do this in the right environment, you will have fun. But the other part of this is about convincing yourself that you're good enough. Remember not to mistake people's greater knowledge base for greater intelligence. I would say that if you find yourself curious about the way living things work, then—and if you find yourself able to readily understand it—then this is a career for you.

4. How do new epidemics arise? (6:48)

Good morning. I have to say I'm very grateful and relieved to see that you've all come back. In my last lecture, we talked about how scientists identify new human pathogens, and I promised at the time to tell you about how new epidemics arise. Where do new epidemics come from, and how can we understand how they arise, how they spread, and why they go away? Now, at first blush, I'm sure you're thinking that this is an impossible question, that there can't be any single unitary answer to this question because there are so many different microorganisms and they produce so many different diseases and they're spread by so many different routes. Are there any general principles that help us to understand where epidemics come from? And the answer is that, at least for viral epidemics, there are some principles, and I suspect that these apply to bacterial disease as well.

5. New diseases arise from disruption of virus-host equilibrium (7:47)

So what I want to do this morning is to discuss the subject of how epidemics arrive in the context—arise in the context of one particular notion, which is that for a new viral disease to emerge, something has to happen to disrupt a previously established equilibrium, or balance, between a virus and a host. That's the general principle here. Now, there are, in fact, two broad ways of thinking about how such a disruption might come about. One way— and this is the way that we typically think as microbiologists—is that genetic change in the virus itself occurs, that some change in the genome of the organism changes its biological properties and allows it to access a new host species or a different organ or tissue within a host. This is an idea that I think is scientifically very accessible. It's also the main idea that drives Hollywood movies about epidemics, like "Outbreak" and other movies in which a mutation occurs that creates this super organism all at once that takes over the world, and it requires Dustin Hoffman to intervene and save everybody. But, in fact, genetic change in the organism is important, and we'll discuss it. But something that's more subtle than that and less obvious initially is that other changes, absent changes in the virus, changes that occur in the environment of the virus that disrupt its previously established ecological niche, can also create new epidemics. Some of these changes are natural, particularly climate changes, but other changes are man-made, and we'll talk about some of those towards the end of the talk.

6. Genetic changes in viruses: Mutation and recombination (9:27)

But let's begin with thinking about genetic change in the virus itself. Next slide, please. I want to discuss two different genres of genetic change that can happen in a virus because they each make contributions to the evolution of new epidemics. Perhaps the most obvious form of genetic change in a virus is a simple mutation, base changes that change in the DNA or RNA genome of the virus that result in changes in the amino acid sequence of its resultant proteins that confer new properties on the virus. But, in addition, viruses can also undergo recombination with one another. Viruses, particularly if they are genetically related to one another, can exchange genetic information between each other. That can also confer new properties on the recombinant virus that emerges, and this also goes on in nature, as we'll see.

7. High rate of mutation in RNA viruses (10:16)

Let's begin by talking about the simpler case of mutation. Now, it turns out that not all viruses have the same mutation rate. Unlike bacteria, in which mutation rates are generally comparable among the different species, as Brett alluded to last time, different virus families have different rates of mutation. In particular, viruses whose genomes are RNA typically have very high mutation rates. Why is this? Let me give you as an example of that the retroviruses like HIV. It's been estimated that with each replication of viral RNA, one mutation is generated. One error in copying viral nucleotide sequences is made in every 10,000 bases or so. Now, since retroviral genomes are on the order of 7,000-10,000 bases in length, that means that, on average, just about every newly made or every other newly made genome in the replicative cycle will harbor a mutation, a staggering amount of genetic variability.

8. Mutations that do not change the amino acid sequence (11:15)

A lot of these mutations are silent. Some of them don't change the amino acid sequence. Can anybody think of a way in which a mutation can occur and not change the amino acid sequence in a protein? - Yes. -It's in one of those sections that is noncoding. Mutations in noncoding regions won't be expected to change the amino acid sequence. Any other ideas? Yeah. Some codon sequences code to the same amino acids. Right. Some amino— the genetic code is degenerate. There are more codons than there are amino acids. Some amino acids are encoded by multiple codons. If you change one of those codons to another codon encoding the same amino acid, you'll get no change. That's one way in which a lot of these mutations can be silent. Some of them change one amino acid to a very closely related amino acid, leucine to isoleucine, for example. That doesn't often result in a very large change in the function of the protein. But other changes will change the protein. Those are important causes of potentially new biological activities that mutations can arise.

9. Why do RNA-based genomes have a higher mutation rate? (12:15)

Now, why is it that RNA-based genomes have these higher mutation rates, higher than we would expect for a DNA-based genome like a bacterium or even our own human cells? I think the most informative way to think about that question is, why do DNA-based genomes have a relatively lower mutation rate? The reason for that is that most DNA polymerases have an activity, have a biochemical activity, that removes aberrantly incorporated base pairs and that checks to see— like the spell checker program of a word processor or copy editor at a newspaper—that if errors are made, typos are made, that they are edited out, and this is referred to as a proofreading or editing function. Most DNA polymerases have that activity, including our own polymerases. Now, mammalian cells don't have RNA-dependent RNA polymerases. So when viruses that have RNA genomes enter the cell, they have to encode, or bring in with them, their own RNA-dependent RNA polymerases, and these enzymes, for reasons that we're not entirely certain of, lack this proofreading activity and therefore don't have this editing function. It's like typing without a spell checker. Errors accumulate, and we're going to see some examples of that in a

minute,

10. DNA proofreading mechanism (13:36)

but let me tell you a little bit about how the proofreading function works in a DNA polymerase. So here's the familiar DNA replication scheme in which parental strands unwind and daughter strands are laid down on parental templates. Here's the template strand here, which is shown as a run of A's. Here's the newly growing strand. You know that nucleotides are going to be added to the 3 prime hydroxyl group. Normally, of course, if "A" is the template, "T" would be the nucleotide that would be added. But what would happen if by error the polymerase incorporated a "C" residue here? Well, of course, stable base pairing with the "A" residue would be impossible. This mispaired base would result. And DNA polymerases have the ability to get rid of that by a nuclease activity that they have that proceeds in the 3 prime to 5 prime direction and can excise this base and sometimes even adjacent bases and give the polymerase a chance to start over, just as you would on a word processor if you hit the delete button and you get a chance to remove the error you just incorporated and type again and try to get it right the next time. That's the main activity that proofreads genetic information in DNA-based genomes. But RNA-based genomes lack this biochemical activity, and that's the central reason for their very elevated mutation rates.

11. Consequences of an elevated mutation rate (14:55)

What are the consequences of these elevated mutation rates? There are, of course, many. In HIV, I think all of you are familiar with the fact that elevated mutation rates give rise to variants of the virus that can be resistant to anti-viral drugs. This is a huge problem in the therapy of HIV infection. Today I want to talk about similar changes that occur in influenza virus that have very great impact on the natural history and origin of new epidemics.

12. Influenza virus: Protein and genomic structure (15:23)

So let's talk about influenza virus for a second. Influenza virus, like HIV, is an enveloped RNA virus. Its outer envelope contains two proteins that are exaggerated here for effect—hemagglutinin and neuraminidase. I want to particularly draw your attention to the hemagglutinin protein. This is the envelope glycoprotein of influenza, and it's responsible for binding to the cell surface receptor and mediating entry into a cell, as I showed you last time on the animation. Neuraminidase is another important surface protein that has many functions but is thought to be involved in accelerating the release of virus particles from the cell surface and making that process more efficient. I want to point out two other issues here. The virus also encodes an RNA-dependent RNA polymerase that is made up of 3 polypeptides and an accessory protein that helps bind the template. But the most important thing I want to point out in this context is that the RNA genome of influenza is a little unusual in that it is not a single RNA molecule, but it is a constellation of 8 RNA segments. 1 through 8. In fact, each one of these RNA segments encodes a single viral protein, more or less, so that it's one gene, one protein in influ—one RNA, one protein in influenza. So keep that in mind that the genome is segmented. That turns out to be very, very important for a story that I will tell you shortly.

13. Pathology of influenza (16:50)

Now, influenza virus causes the disease influenza, which is, as you all know, a respiratory infection. It's principally an upper respiratory infection involving the pharynx, the trachea, and perhaps the proximal bronchi. In you and me, this disease is a self-limited condition. It lasts about a week. People can be uncomfortable, but it does get better because of the supervision of the immune system, antibodies to hemagglutinin and neuraminidase, and also cytotoxic "T" cells and other effector cells help to eradicate

the infection and provide it a degree of immunity to a subsequent infection. We'll come back to that in a second. Now, influenza can be a much more serious disease in the very young or in the elderly, and especially in people who have underlying lung disease, emphysema, bronchitis, congenital syndromic heart disease, and in those groups, influenza can be much more severe.

14. Influenza epidemics caused by antigenic drift (17:48)

I think most of you are aware of the fact that epidemics of influenza occur all the time. In fact, they occur with great regularity just about every winter, between November and late February, early March, being the peak periods every year for an epidemic of influenza, and I want to talk a little bit about this epidemic behavior of influenza. Every year or so, there's an influenza epidemic. Now, I just finished saying that influenza infection is terminated in an individual by the immune system, and the same thing is true in a population. When enough immune individuals occur in the population, it's difficult or impossible for the epidemic to continue, and it fades away and dies out for the season. But yet, every year, there is the recurrence of an epidemic in the wintertime. How does that happen? The garden-variety epidemics occur because each year, the new flu strains which are related to the ones that occurred last year are slightly different antigenically. The hemagglutinin and sometimes the neuraminidase of the virus that produced this epidemic is a little different than the one that produced the preceding one—genetically related, antigenically related, but a little different, and it's just different enough to evade immunity and allow an epidemic to happen. Those small differences that occur in these annual epidemics are referred to as antigenic drift. They give rise to our periodic epidemics of influenza.

15. Influenza pandemics caused by antigenic drift (19:16)

But then every so often, every 10 to 40 years, a bigger change in the antigenic structure of influenza happens. Wholly new hemagglutinins seem to arise that are totally unrelated to the ones that went before. This is referred to as antigenic shift, and when that happens, you get what are called global pandemics of influenza in which large populations all over the globe are simultaneously experiencing influenza. The reason for that is that there is no preexisting immunity at all to these strains. They are wholly unrelated to the strains that went before. So antigenic drift produces our annual epidemics of influenza. The bugs are still slightly related to the ones that preceded them. There is a limited amount of cross immunity, but not enough to prevent an epidemic from happening, but every once in a while, there's antigenic shift. An entirely new antigenic configuration is presented on the cell surface. Preexisting antibodies can do nothing at all, and there is global pandemic spread of influenza. OK? Now, the question is, how does that arise? What's the genetic basis of antigenic drift and antigenic shift?

16. Genetic basis of antigenic drift (20:27)

Antigenic drift is due to the accumulation of single point mutations or constellations of point mutations in hemagglutinin and neuraminidase that come about from the lack of the proofreading function in the flu RNA polymerase. The net result of that is that preexisting antibodies to these mutant proteins from last year's epidemic don't bind as efficiently to the envelope, and the virus is capable of, to one degree or another, evading immunity, and that enhances its ability to infect an individual and spread from one individual to another. That's what's the genetic basis of our annual flu epidemics, and there's an important corollary to that, which is that, because of antigenic drift, we need to make flu vaccines every year or so to account for the upcoming strains of influenza. This imposes quite a burden on the scientific community. One has to be out there surveilling the early strains that are circulating in time to figure out which--what the nature of the strain circulating this year is so that it can be incorporated into a vaccine that is appropriate for that year. So I think you can see that the lack of an editing function in the polymerase results in a very significant issue for scientists for vaccine production and explains the

annual epidemics of influenza.

17. Molecular structure of hemagglutinin (21:52)

We know a lot about the nature of the changes that are occurring in hemagglutinin as a result of antigenic drift. Here's the hemagglutinin molecule at high resolution. It's a trimer, 3 molecules together on the cell surface. Here's a single monomer of flu hemagglutinin, and this is the region that's involved in binding to the receptor, the globular head of the protein, and in "a" through "e" are shown areas of hemagglutinin in which mutations arise as a result of antigenic drift. So, these mutations allow the viral hemagglutinin to continue to bind to the receptor. They would have to do that. Otherwise, the virus would be dead. But they impair the binding of antibodies that would normally block the ability of the envelope protein to bind to the receptor. That's what we think is going on at the molecular level during antigenic drift. OK.

18. Genetic basis of antigenic shift (20:49)

Now, that's all very well, but how does pandemic influenza arise? What's the genetic basis of antigenic shift, these wholly different hemagglutinins that appear to arise on the cell surface, on the viral surface? Here we have to come back--I have to introduce you to a little nomenclature because it turns out that there are 3 major antigenic types of hemagglutinins that have been recognized among the human influenza viruses, and these are termed H1, H2, and H3. If we take the ordinary case of the annual garden-variety flu epidemics that occur by antigenic drift— Let's take the case of flus that occurred between 1957 and 1967. All of those were due to strains of influenza whose hemagglutinin would be classified as H2. But in '58, '59, '60, and so on, there were slight changes from antigenic drift due to errors of the polymerase that gave rise to variants of flu hemagglutinin that were a little different but all still recognizable as members of the H2 group, OK? Then, in 1968, when I was a freshman in college and contracted this disease, there was a giant shift in the antigenicity of influenza. What emerged was something called an H3 virus, whose hemagglutinin antibodies to H2 didn't protect at all against this, and there was a global pandemic of influenza, which in the world of influenza was considered of moderate severity. But I was flat on my back for a week in that dormitory as a freshman. I would have called it severe. But, in any case, this is illustrative of antigenic shift.

19. Origin of different influenza hemagglutinin types (24:31)

Where did H3 come from? The answer to that came about when virologists began to look at the distribution of influenza viruses in nature. It turns out that, although I've been at pains to tell you that human influenza strains are generally of 3 "H" types— H1, H2, or H3— out in the world of animals, you can find 15 different hemagglutinin types—H1 to H15. The 3 human types are represented there, as are 12 other types, H4 to H15. And the principal animal species that harbor this great diversity of influenza viruses are not mammals but birds, especially migratory waterfowl like geese, ducks, and chickens—domestic and wild waterfowl. All the influenza strains that have ever been identified are represented in this population, and it's thought on that basis that these birds are, in fact, the major repository of the new strains of influenza that exist. Now, OK. How does that influenza viral hemagglutinin get from the avian reservoir to the human reservoir? This is a question that has two components. There's a cellular component, and there's a whole animal component.

20. Animation: Recombination of viral RNA in a host cell (25:51)

Let's talk about what goes on at the single-cell level. How does a gene for influenza hemagglutinin get into a human virus? And I've prepared a small video to show you how this goes. Before we roll the tape, let me just introduce you to the fact that I'm going to show two influenza strains here, one of which has

red genomic segments and one, blue, OK? What you're going to see in the video is that new human hemagglutinin types come about by recombination between two strains. That recombination requires that the two different strains infect the same cell. When they do that, what you're going to see are the two viruses binding to the cell surface receptors as before, enter the cytoplasm, disassemble, and start replicating their genomes. And then you're going to see an important new event happen. Let's roll the tape. You can see particles are going to bind to the cell surface and fuse with the plasma membrane, release their contents, disassemble, and liberate their genomes. Now, let's stop the tape for a second. You know from last time that the next event that's going to happen in viral replication is that these RNA segments are going to be transcribed and translated to generate more viral proteins. The RNA is going to undergo RNA-dependent RNA replication. You're going to get lots of new copies of this subunit. But now, because two different strains have infected the same cell, their RNA subunits will freely admix with one another so that when the time comes late in infection to reassemble into daughter virus particles, subunits from one strain can occasionally be picked up along with subunits of the other strain and packaged into a single virus particle. Let's roll the tape and watch that process happen. That is a form of recombination that results from the mixing of subunits, and you'll see it shortly. So here are the newly replicated genomes. They are going to go out now and assemble with newly made proteins into progeny particles. Here you can see the process happening. If you just concentrate on this particle, you can see—let's stop the tape—that two blue subunits have been incorporated along with 6 red subunits and vice versa over here. You can see that from such a dually infected cell, a whole host of new recombinant viruses bearing different subunits have occurred. So this is a form of genetic exchange that occurs not by crossing over but by swapping whole viral minichromosomes, if you will, OK? And so we can complete the tape now, and you'll see these particles will go out, pick up their envelopes, and go off into the outside world. This will be a mixed stock with all different varieties of recombinants, and those recombinants that do best in nature will survive. So that, at the molecular level—we can terminate the tape now—is where new recombinant strains come from, that is, at the cellular and molecular level, the ultimate source of all these new shifted variants. Now,

21. Where does recombination take place in nature? (28:56)

that's fine as a laboratory experiment, but where does this happen in nature? It turns out that avian strains of influenza don't replicate very well in humans. There's very little direct transfer of avian strands from the bird reservoir directly into man. It happens rarely. In fact, there was an outbreak of influenza in Hong Kong in 1997 that you may have heard about in the news in which there were a dozen or so cases in human beings that appeared to result from a direct transfer from birds into humans, but that's a rare occurrence. Avian strains don't grow very well directly in humans. They do, however, grow very well in pigs. For one reason or another, that's never been wholly explained. Pigs are mammals, and porcine influenza strains actually turn out to grow rather well in man and vice versa. So humans and pigs can trade influenza strains back and forth. So the route by which new hemagglutinin subunits enter the human population is not generally a direct one. Rather, the avian strains infect pigs. This is particularly common in societies in which agriculture is practiced in a mixed setting in which pigs and ducks are all raised on the same farm. You know "Old MacDonald Had a Farm?" If he had pigs and ducks on the same farm, then old MacDonald could also wind up with influenza. So transfer to the porcine reservoir occurs. The mammalian strains in the porcine reservoir can also infect human beings. So pigs are referred to in influenza virology as the mixing vessel in which the ultimate reservoir of subunits from birds are transferred to human beings. OK? So that's a particularly striking example of genetic exchange in the pathogen—recombination giving rise to a wholly new hemagglutinin subunit that can then admix with other mammalian subunits that confer efficient growth in human cells, and the progeny of that can be transmitted to humans, and now you have a virus that, unlike most bird viruses, grows very well in man, but also has a new and wholly different antigenic subunit on its surface of hemagglutinin that is resistant to previous antibodies and therefore can grow very well and spread in a pandemic fashion. OK.

22. Environmental changes can cause new epidemics: Hantavirus (31:18)

So those are two examples in one virus of how genetic change in the virus can create new epidemic strains. But I was at pains to tell you at the beginning that not all new epidemics of human disease occur as a result of genetic change in the virus. Now I'm going to show you a few examples of how genetically unaltered viruses can nonetheless produce new epidemics as a result of a change in the environment that alters their normal ecological niche. You may remember in 1993 headlines like this, in which a new disease, a severe disease of respiratory failure occurred on Navajo reservations and surrounding communities in the American Southwest. This disease we now know is caused by a virus called a hantavirus. It occurred in the Four Corners region of the American Southwest. It was characterized by a rapid progression of high fever, pneumonia, and respiratory failure. A new pathogen discovery effort that proceeded along the lines of what I told you about last time—it involved both antibody testing and genomic based discovery and traditional viral culture—rapidly yielded the result that—what emerged was a new member of a family of viruses than had previously been known. The hantaviruses were discovered during the Korean War on the Hantan Peninsula of Korea. They're viruses that exist in a rodent reservoir. They're excreted in rodent urine, and in the few cases that had been described earlier, most of these viruses produced in humans not respiratory disease, but kidney disease. We knew something about the hantavirus family. No one had ever identified a hantavirus that was capable of producing respiratory disease before. This hantavirus was named the sin nombre virus, which is Spanish for "no name." And so it was clear that this was a hantavirus that had not been seen in human medicine before.

23. What environmental change was responsible for the hantavirus epidemic (33:25)

Where did this virus come from? Well, it turns out that like all hantaviruses, this is a virus that circulates among wild rodents—deer mice and others. In fact, by testing archived specimens of serum from deer mice as long ago as-- from many years ago, it was clear that hantaviruses have existed—this particular sin nombre hantavirus has existed in the mice of that region for a long, long time—many decades. The infection in mice is asymptomatic. They shed their virus in urine like most hantavirally infected mice. Humans get infected by contact with rodent urine. If there has been no change in the virus and the virus has been hanging out in these rodents for that long, how is it a new epidemic arose? It turned out that the basis for this was a disruption in the normal ecological niche of the virus. It happened that in the two previous years, there had been an exceptional amount of rainfall. As a result of that, there was an abundance of vegetation, including the pinon nut, which is the main food source for these deer mice. As a result of the overgrowth and wide availability of food, the deer mouse population expanded very significantly during those two years. And they were able to invade the domiciles of the people living on the reservation. Which, as you may know, is an environment of significant poverty in which there is a significant problem with rodent infestation of homes. When there were more rodents, wild rodents in the community, there were more in the homes, more exposure of the human beings. Presto, an epidemic, and, in fact, without any genetic change in the virus. So this is a really powerful illustration of how things as innocent as heavy rainfall can change and create a whole new epidemic that had never been seen before. This epidemic subsided when normal climatic conditions were restored as well as the advent of more stringent rodent control in the homes.

24. Human migration affects epidemic patterns: Smallpox (35:16)

There's a very strong example of how ecological changes can create an epidemic. You shouldn't have the idea that all new human epidemics come from an animal reservoir. There are lots of viruses that will grow only in humans, like this one, smallpox virus, that produces this devastating systemic disease with severe skin lesions and also internal infection of many organs. And smallpox has been known for a long time. This is a disease which has now been eradicated by vaccination. But smallpox, being limited to a

human reservoir, has a history that is very well understood. It is a potent illustrator of the capacity of human migration to change epidemic patterns. It turns out smallpox virus has been resident throughout evolution in human beings in Europe and also in Sub-Saharan Africa. But smallpox was unknown in the Americas, in North and South America, until the arrival of European colonists and their African slaves. When those people began to arrive in North and South America in large numbers, they brought with them smallpox virus to the Americas. When smallpox reached these Indian populations in North and South America, who had previously never been exposed to smallpox, severe epidemics of smallpox broke out among the natives in North and South America. Here's a little account from a colonial governor in Plymouth colony in North America who said the Indians, "fell into a lamentable condition "as they lay on their hard mats. "The pox breaking and mattering and running into one another. "Their skin cleaving by reason thereof "to the mats as they lie on. "When we turn them, a whole side will flay off at once. "Most fearful to behold. "And they being very sore, what with cold and other distempers, "they die like rotten sheep." This disease took on a fierce countenance in the Indian populations—not just in the North American Indians, but also in the Aztec and Mayan populations. As a result, there was a massive mortality among these tribes, especially in Latin America, and it is believed now that it was very unlikely that the conquistadors who arrived with several thousand admittedly well armed soldiers could have overpowered the fierce warrior cultures that they encountered, which numbered in the many hundreds of thousands, had it not been for the tremendous decimation of the indigenous population as a result of smallpox.

25. Why was smallpox so much more severe among Native Americans (37:52)

So, smallpox has had a huge impact on human history. Now, so there we can see an example of how human migration changes the pattern of an epidemic disease. But can we understand why it was that smallpox appeared to be so much more severe in the Indians than it was in the White and African migrants who brought it to them? And the answer is we have the beginnings of such an understanding.

26. Lesson learned from rabbits in Australia (38:15)

It comes from studying not human smallpox, which has been eradicated, but a pox virus infection of this species, the European rabbit. Now, rabbits were brought by—Rabbits do not naturally exist in Australia. They were brought by English settlers to Australia to use for sport in hunting. And when the rabbits arrived in Australia, they encountered rabbit nirvana. There are no natural predators for rabbits in the continent of Australia. As a result, they did what rabbits do, which is to multiply like rabbits. Soon the entire countryside of Australia was denuded by rabbits. Those of you who have pet rabbits know that they devour large amounts of vegetation. This is an old slide that shows a fence running down the middle of a farm. On this side of the fence are where the rabbits are, and you can see, there is no vegetation, where you see abundant vegetation on this side. It is for this reason that rabbits are the sworn enemies of Australian farmers. Australian farmers have been pressing the Australian government to do something to get rid of these rabbits that have become major agricultural pests.

27. Myxoma virus as a rabbit-control agent (39:19)

Now, it happens that rabbits have their own pox virus, called myxoma virus, which produces a devastating immunodeficiency and systemic infection and hemorrhagic fever. This is a moribund rabbit about to die from myxoma. And myxoma has one other property, which is it is highly specific for rabbits. No other mammalian species is known to be infectable with myxoma virus. So the bright idea occurred to certain virologists and many farmers in Australia in the fifties—Hey, why don't we infect some rabbits in the laboratory with myxoma virus and then release them into the environment? This is the sort of uncontrolled biological warfare that we don't engage in generally anymore. This happened in

the fifties in a wholly different social climate. I don't want to defend the experiment, I just want to show you what emerged. From this we have learned quite a lot about the issue of viruses newly entering populations that have not seen them before.

28. Less virulent myxoma strains emerge and maximize the spread of disease (40:21)

I need to tell you one thing, which is that myxoma variants can be classified according to how virulent they are, how severe the disease they produce, and how rapidly they produce it, with grade I virulence being highly virulent. 100% of the animals die within—in less than ten days. Grade IV being isolates that kill less than 50% of the animals and take a long time to do it. And grades II through V are in between. What was released into the Australian Outback in 1950 were rabbits that were bearing highly virulent grade I myxoma virus. As recently as four years later, if you went back out into the field and collected myxoma isolates, most were of milder grades—II, III, and IV. And by 1956 you started to have significant numbers of grade V isolates, which are—although they can still produce disease, kill only half the infected rabbits and take much longer to do it. What does this say? It says that during the spread of the epidemic, mutant strains of the virus were being selected for that were less virulent. Can we understand that? Yes, we can. It relates to something that Brett said in passing in the last hour. What evolution is operating on is not disease. Disease is incidental. It operates on spread. What myxoma virus wants to do is spread as much as possible. If a grade I myxoma virus infects a rabbit and that rabbit is moribund in 48 hours, it's not going to go out and socialize with other rabbits and spread the virus. Animals have to be carrying infectious virus and be well enough to go out and disseminate the virus in order for that virus to spread. The net result is grade I myxoma virus doesn't spread very well. The ones that spread best are the ones that allow the rabbit to go out and associate with other rabbits, be well enough for a while to spread infection. We can see that over time when a virus has been residing in a population for a while, there is a selection, oftentimes, not always, but oftentimes for less virulent viruses that can spread better. Now, remember, the virus is not interested in disease. It's only interested in spreading. But when disease interferes with spread, there is a selection against pathogenicity and for lesser grades of virulence that allow dissemination. That's one key fact that explains why viruses that have been in a population for a long time have a lesser degree of virulence.

29. Natural selection results in minimized susceptibility to disease (42:48)

But you have to also realize that the host animal, the mammalian host, also undergoes evolution during this kind of spread. Because when a lot of susceptible animals die, the survivors tend to be less susceptible. Here's a good illustration of that from the myxoma case. What was done here was to go out into the Australian Outback, into populations of rabbits that you knew had been exposed to varying numbers of epidemics of myxoma. No epidemics, two, four, or seven previous epidemics. You don't want to go out and test all the rabbits in this environment because a lot of them have survived and recovered from grade V myxoma and they'll have antibodies that will make them immune. So what you do is you go out to these populations and you look for the ones that have no antibodies. These are ones that have never been infected. They are residing in a population where myxoma has been present, but these animals have, for statistical reasons, escaped infection. Now you want to ask, how susceptible are these non-immune wild rabbits from these different populations to laboratory inoculation with myxoma? What you discover is that if they have never seen the virus before in nature, most of them have a fatal outcome. But if these animals have been through—let's take this case where their seven previous epidemics have blown through the colony—what you discover is that most of the survivors, even though they themselves as individuals had never had an infection before, have a mild outcome. What does that say? It says they harbor host chromosomal mutations in their genomes that confer relative degrees of resistance to the pathogen,

30. Comparing the myxoma virus in Australian rabbits and smallpox in Native Americans (44:26)

just as the Europeans and Africans who immigrated to America had been the result of an evolutionary selection for resistance to the pathogen, while the Indians populations who resided here were wholly susceptible. I think the myxoma experience has given us a way to understand what was going on in Plymouth and in Mexico when the English and the Spanish settlers arrived. Virus host co-evolution. As you might imagine, this virus host co-evolution resulted in the abject failure of myxoma to control the rabbit population. And this is Time Australia, the Australian version of Time magazine from 1995 or so, and you can see the rabbits are back and the farmers are still trying to figure out how they're going to deal with this.

31. What forces will shape the epidemics of the future (45:11)

What does the future hold? Can we take these general precepts and make some guesses about what kind of epidemic diseases we're likely to see or what forces will shape epidemics in the next part of the millennium? I think what we can see is we can't predict genetic change in the pathogen. That's going to happen according to its own molecular logic. But we can start to see changes in the environment that we as human beings are doing that will have—we can strongly predict—an impact on new epidemics. We are seeing a global wave of urbanization throughout the Third World-- people leaving rural farms and migrating into giant cities—Mexico City, for example. New Delhi—teeming with people, where there's close crowding and poverty and lack of sanitation. We're seeing a globalization of international travel, that Joe Perpich mentioned in the introduction, which is capable of bringing things from one remote corner of the globe to another in no time flat. And finally a globalization of commerce. In the remaining minute or two, I want to tell you about one little anecdote that I think illustrates how subtle these societal changes can be and still result in disease.

32. Subtle changes affecting spread of diseases: Dengue fever and West Nile virus (46:21)

It turns out that one of the things that global commerce has wrought is that there's a global commerce in the recapping and reuse of tires. Tires in the Third World are shipped—stored in dumps and then shipped on boats back to America for recapping and resale. These dumps occur in the open air and rainwater accumulates in these tires—the perfect pool of stagnant water to breed mosquitoes. What's happened over the past decade is that the mosquito vectors for a viral disease called dengue, which has been eradicated in America until recently, the mosquito vector has been reintroduced into the American southeast as a result of the shipping of these tires back to America. Now dengue has not yet reappeared as a disease in America, but the potential for it to do so now exists because dengue exists in Cuba and other Caribbean islands 90 miles from our shores and the mosquito vector has been reintroduced into America. So this is an illustration of what we might anticipate. I think another live example of that is the recent appearance of the West Nile virus, a mosquito-borne virus that was thought to reside only in Africa in Manhattan and upstate New York very recently. So microbes are on the move and they're on the move in no small measure because of these changes in society and the economy.

33. Conclusion (47:32)

So what do we conclude? We conclude that infectious diseases as a group will never disappear. We are living organisms living in a sea of microorganisms, and infection is part of human evolution. What we hope to do is to control individual infectious diseases through research in microbiology and infectious disease, but rational control of them is based on the kind of science that Brett and I have been talking about. But science alone will not do the job. We have to understand that societal factors, economic factors, are a major part of what's going to happen with epidemic infectious disease in the years to come. With that I thank you all for your attention and invite your questions.

34. Student question: How do DNA viruses mutate? (48:13)

Let's start with a question from the house. Yes? Would it be unrealistic to attempt to create a vaccine that would be a broad vaccine that would be able to take care of any mutations? The question is, could we imagine developing an influenza vaccine that would be more broadly reactive and cover all the influenza strains that are out there? In fact, there was recently a paper published in Nature Medicine, a leading journal, about that very subject. It turns out that an HHMI investigator named Bob Lamb, many years ago, showed that one of the influenza subunits, the M protein, is invariant or largely invariant among strains and immunity to it does exist and helps confer immunity to influenza. So there is some desire to try to see if we can base immunity to influenza on something other than hemagglutinin, which is so variable, and might be one way to approach what you're suggesting. It's not clear that it will work, but it's something that one is thinking about. And that's an insightful question that you asked.

35. Student question: Can different species of bacteria and viruses trade genes? (49:19)

Let's go to one at Penn State. Go ahead, Penn State. Hi. My name is Jeff. I'm from Hershey High School in Hershey, Pennsylvania. My question is, you have discussed the proofreading ability of DNA. If a virus has this biochemical activity, how are mutations formed? Viral—DNA viruses that operate by DNA dependent polymerases often use host DNA polymerases as their machinery. So they have the low error rates that are associated with the host enzymes. How do they have mutations? Even the host enzyme makes mistakes once in a while. The editing function isn't perfect, it just operates to improve the efficiency of it. Just like your typing isn't perfect. You may proofread a draft and then discover the next day that there still are one or two typos. Not as many as you had originally, but even the proofreading machinery has an error rate, and that's where we presume the residual mutations come from in DNA based genomes.

36. Student question: Can different species of bacteria and viruses trade genes? (50:18)

Anybody else in the audience? Yes. My name is Brittany. I was wondering if—can different species of bacteria switch the resistance? Can they trade resistances? Can different species of bacteria trade resistance among one another? The answer to that is yes. They—there is cross species conjugation of the type that Brett showed you on his animation yesterday between different gram negative rods, for example. That exists in nature in the intestinal tract. And it's thought, as Brett alluded to, that many of the resistance genes we have identified in one species originated initially in another species and were transferred. So that definitely happens. And in the laboratory you can carry this to quite an extreme. There is a scientist at the University of Oregon who some years ago showed that gram negative bacteria could even transfer genes into yeast if you engineered it just so in the laboratory. We doubt that that happens very much in nature. That kind of broad spectrum exchange definitely occurs among bacteria. How widely can viruses share their genes? In general, the kinds of recombination processes that we've talked about occur only within viral families. So retroviruses can share genes with one another, herpesviruses with one another. It is not so clear that viruses of entirely different families can efficiently share genes. One thing that definitely happens is viruses of several families can pick up genes from their mammalian host. In the K.S. virus that we work on, which is a herpesvirus, I didn't mention this yesterday, but there are half a dozen or more genes that have no homologues anywhere in virology but which are homologous to genes that we know exist in the cell. Those genes have been somehow captured by the virus from the cellular genome. Retroviruses are famous for doing that. And when that happens they often have powerful new activities that change their biology. So, there is that form of cross-species exchange in viruses.

37. Student question: How do researchers and health organizations share information? (52:17)

Let's go to Miami now for another question. Go ahead, Miami. Hi. My name is Janine. I'm from Miami Northwestern Senior High School. I would like to know, how is information about new viruses shared between researchers and health organizations? How is information about new viruses shared between public health officials and virologists? I think influenza offers a very nice example of that. Because every year, professional influenza virologists have to be out characterizing field isolates. So practicing physicians and public health organizations from various governments go out and forage early in the summer and fall, ahead of the flu season, and often in the spring even, for new isolates which they then ship to reference laboratories to determine their antigenic structure and characterize them. There is a case where there is active surveillance by governmental and private organizations that is then translated into laboratory science. I think what we are— if you take my drift now from the theme I put forward, we need more of this. We need more governmental surveillance all over the world of isolates of many different types so that we can be one step ahead of the next epidemic. Right now we practice largely in a reactive mode in which as soon as we identify a problem, we try to respond to it. Is there some other way we can survey more broadly among more virus and microbial families? That would require a major partnership among different governmental organizations that are dedicated to public health as well as to laboratory scientists. That so far is just being thought about now.

38. Student question: Are some regions of the influenza genome more mutable? (53:56)

Let's take another question from the house. May I ask the in-house speakers to speak up a little bit so you can be captured on the mikes. Go ahead. Georgetown Day School. I was wondering, you said certain areas of the influenza virus are more susceptible to mutation than other areas. I was wondering what makes those areas more susceptible to mutation and how can that knowledge help us in defeating the virus? The question is, are some regions of the influenza genome more susceptible to mutation than others? I actually didn't say that, but you might have gotten the impression because I was dwelling so heavily on hemagglutinin and neuraminidase. I dwelt on those subunits because they are principal targets of immunity and change there allows one to escape from immunity. There is also quite a lot of mutation going on in the other subunits. I didn't dwell on it because it is not directly related to the subject of immunity. However, since you asked, it turns out that when you look at a very high level of resolution at the nucleotide sequence of some RNA viruses, there sometimes is biased mutation according to the different sites. There's a phenomenon in measles virus called biased hypermutation in which certain segments of a particular gene appear to undergo much more mutation than other segments. There's a—there are very complicated biochemical mechanisms at work there. So I think at this level I just want to say that mutation to a first approximation can be thought of as random across the whole genome and that selection by the immune system is important in creating new epidemic strains. You are right. When you look at a high level of resolution you can see the mutation rate is not perfectly uniform across every nucleotide, and there are complicated biochemical reasons for that. Ok. I think we are—I'm getting a message that we have to stop. I would be happy to take from the local audience questions at the podium afterwards. Again, thank you all for your attention and for your superb questions.

39. Closing remarks by HHMI Vice President Dr. Joseph Perpich (56:02)

Thank you, Don and Brett, for a superb series on our world and the world of the microbe. As we learn more about the information carried in our own genes and those of microbes, we understand more as we have learned today about the delicate balance that exists between our two worlds. Don Ganem and Brett Finlay and their colleagues, in science, medicine, and epidemiology are in the forefront of advancing human health in the 21st century, as you saw today. From their work, a small step in understanding the world of the microbes, either friend or foe, may often mean a giant step for humankind. Stay closely tuned to the latest breaking news from this new frontier. Now the Institute's president, Dr. Purnell Choppin, will now close this lecture series with brief remarks taped prior to his leaving to attend the

1999 Nobel Prize ceremonies in Stockholm.

40. Closing remarks by HHMI President Dr. Purnell Choppin (

Thank you, Joe. I recorded this message before traveling to Sweden, where one of our HHMI scientists, Guenter Blobel, will receive this year's Nobel Prize in Medicine. Perhaps someday one of you in the audience will be here receiving that prize. I want to say a personal thank you to Don Ganem, Brett Finlay, and to all of you for watching. I also want to take this opportunity to express my appreciation to Joe Perpich, Dennis Liu, Jill Conley, Ann Sutherland, and everyone else who worked so hard to make this series successful. Next year the Institute's new president, Tom Cech, who gave these lectures in 1995, will take my place at this event. I hope you will join us then. For now, from Stockholm and from everyone back at the Howard Hughes Medical Institute, I wish you the happiest of holidays.