Norton Zinder.

Unlike most thesis presentations, we are not going to have a question period. Now, I can't possibly introduce myself. What I'll do instead, is give you my pedigree. Genetically , that's more important. All right.

Because we're a very tight knit group here today, Ed Tatum's three students are here -- Josh, Ed Adelberg and Barbara. I'm Josh's student so I have a father, an aunt and an uncle here today and that's my scientific pedigree. We're pretty close.

Now, I arrived in Josh Lederberg's lab in early July 1948 and once again, Francis Ryan had intervened. He was my advisor at Columbia and he had sent me to Joshua who had just gone out to the University of Wisconsin as a young assistant professor. And I mean young, Josh was 23 and I was 19, I think at the time. An interesting time. It was rather remarkable, now that I think about it. Josh put me to work looking for mutants and that's exactly what I do to new students in my laboratory these days. The first thing they get to do in the laboratory is to look for mutants. There's only one difference. In 1948, there were no ways to get mutants or bacteria. In real ways, anyhow, now there are enough ways, in fact, now you really don't need a graduate student.

You have a machine that does it for you for the most part. And there was a technique that I was given to use and that was to plate E coli on a minimum medium and you let them grow up so a few hundred colonies per plate, so you mark every colony. This in the University of Wisconsin -- in Wisconsin, it's a hot summer. Everything is wet and hot. And then you pour over a layer of complete medium, on top of this, and anything that comes up afterwards, obviously is a mutant that has growth factor requirement. Well, by the beginning of August, I was exasperated. And I can't say what Josh must have felt by then because I had not isolated a single mutant. But everything was contaminated. I should note that I never had a course in microbiology when I arrived and the only lab course I had was in Columbia -- it was organic chemistry and physics -- but we just put electric wires together back in those days. So I didn't even know much about a pipette.

In early August of 1948, Josh hands me a paper by a fellow named Holly, from the Proceedings of the Society of Experimental Medicine and this paper says that the drug, penicillin, kills only growing cells. Now, well I'm not very good in the lab but I'm not totally stupid. I understood immediately what that would mean but I didn't have the vaguest idea of how to do the experiment and Josh taught me at that point what's called a reconstruction experiment. And that is you take a culture, which has some genetic markers already on it and you make a mixture of the culture with other markers on it and you make a mixture and put them in the presence of penicillin and a minimum medium and see whether or not you get a differential killing effect of those that can grow from those that cannot grow. And, EUREKA, I did an experiment on August 15, 1948. It's well marked in my notebook. And the experiment is shown on the slide. And this is the actual data from the experiment that the colonies on the petrie dish are lifted off onto a piece of filter paper and I should just note that at this particular point, if you're doing this kind of thing, this is a lab technique and I don't know where it came. It was there when I got there. And it replicating far behind . Well it was far behind. It was three years before the replicating technique was developed in the laboratory. Because this is replica and it's not without the plating. So I made a mixture of +'s, those are from fermented sugar -- they're black -- and -'s, those are the little white ones. And the ratio of 90:1 is the actual ratio. You can see this was torn out of my notebook so we could take the picture. It's going back; it's rather precious to me and these had a growth factory requirement. The fact of the classical thiamine lucine line. This is Lac + strain. Put them in a minimum medium at about a 10:7 millimeter, add 300 units of penicillin , 2 hours later, (next slide please). It's all over. That's what you call a one shot experiment. All you

have to do is look. You don't even have to count. So I love those kinds of experiments. Now the ratio is now 1:20 and there's been an 1800 fold increase in the number of mutants in 2 hours of incubation in the presence of penicillin. At 6 hours, the data says there's an infinite increase. That means, I couldn't find any Lac +'s anymore.

If you will now look at my notes, within a few weeks of September(this was done in August), there are hundreds of new auxotrophic mutants of bacteria available in the laboratory. And this is a very important technique because it's what I am going to use to develop my thesis.

I'm going to skip to January, 1950. In late 1949, Josh receives in a diplomatic pouch from Sweden, a series of salmonella type strains. And they fall into 22 categories on the basis of phage typing. And in my charge, or my potential thesis, is to prepare a set of complimentary mutants so that you can cross every one of these, pair-wise. Without the penicillin technique, it's a sad joke; with the penicillin technique it is not impossible. At that point I set up (I guess if that was the Computer Era, what would you call it -- a set of macros). There are four procedures I decided upon for doing the penicillin treatment. After all you can vary the amount of mutagenesis. In those days, it was radiation. You can vary how long they grow after the phenotypic expression. You can vary the amount of washing. You can vary the amount of penicillin. A lot of variables. And for some reason for which I cannot tell you at all, there are four procedures which are used. And then if you look in the notebook thereafter, it says Procedure 1 worked/didn't work. How many mutants I got from Procedure 1; how many I got from Procedure 2. Why I chose those particular conditions and their relative parameters is still a marvelous mystery to me. I should say something which I should have said before -- this was the first time that I read through, because of this meeting today, my notes from 1948 through 1952 when I left the laboratory. I've looked at them occasionally for odd bits of experiments but never gone through the sequence of events. If nothing else, I certainly learned a great deal the way we thought those many, many years ago, I should say.

By June 1950, which is not terribly many months, remember I'm going to a real graduate school and taking lots of courses. By June 1950, (I'll have more to say about that too), I note that all self-courses failed. That is, every course, of strain by strain. I had complimentary pairs of mutants. They all failed. And I guess, out of desperation, because there's no theory now, I start crossing in-between strains. And remember, Josh , and I believe that he believed very seriously that coli was homothallic and that the system is homothallic. And, in fact, if I got a cross to work between two different strains, it would already have been different than K12.

But I started to do that and in the summer, in the first nibbles of crosses that worked. There are a lot of crosses to do -- actually 400 -- 200 if you only do them one way at a time. And they were done slowly and analyzed because there are always contaminants and what not that come up that give you false leads and false positives. But they were beginning to show some signs that some of the inter-crosses were actually working.

So we now come to the Fall of 1950. Now the Fall of 1950 is one of the hardest periods of my own life. If my wife was here, she would tell you that she was about to deliver a child -- which she did on October 22nd, my first child. I was taking human pathology because I was going to get a degree in microbiology and not in genetics, for a variety of reasons for which we needn't go into today. But it's very difficult to take human pathology if you have not first taken human anatomy. It's hard to know what something is wrong with it if you don't know what it looks like when it's right. And it was a rather difficult course at the time.

And in the meantime, on October 5, as luck would have it, I did a cross between two of the strains

Lillian was the Swede who had done the classification. Sorry I didn't mention his name before. Gave recombinance, that looked like color recombinance a frequency of 10th to the minus 50 (the plate was just loaded with them). It was really exciting. And so we started to look around and slowly, slowly (it takes a while) for things to get into your head. It is clear that the important part is to cross things to type 22.

So now it becomes more like color. As Josh said before, he not only had the ++++ phenotype; that is, the 4 +'s if there was two pairs of markers being selected. But he threw in a Lac marker, and made one black + over black - or something else, etc. etc. etc.

I alternately threw in nine markers, none of which segregated. Not one of them segregated, other than the marker that you selected. And what was even more remarkable, you could always set up the cross in such a way that you select another pair of markers in the same strain. And they would give you 10:-5 recombinants and all the other markers wouldn't segregate. And there was one further point. The markers that wouldn't segregate were always those of LT22. It was an asymmetric selection.

At this point, I guess it was probably Josh's idea, I'm never really sure. You can credit most ideas in the lab to Josh -- he decided to find out whether or not it required cell to cell contact for the mating to occur and we did the famous U-tube experiment. This was an experiment which was first done by Bernie Davis, with E coli K12 -- the story Josh told you about -- in which you mix in separate arms of a tube two different cultures and they have a stinted glass sterile filter in between and then you flush it back and forth and see whether or not you get sex.

Now let's have the next slide please. This is just a control to show you that bacteria don't grow when they are mixed.

Next slide. It's on your program. That's why I'm showing it to you. This is an experiment with LT2 by LT22 and the next slide is the U-tube -- the Scientific American article -- that's the U-tube, shaped like a U, has a stinted glass sterile filter here, the two arms on the side where you can apply suction, pressure and flush the fluid back and forth, and you grow one bacteria on one side, another bacteria on the other side. And I'm not going to go through this one .

And the next one is a diagram of what we should have (next slide please) understood what happened. We did understand. That's what I mean about context. As I said before, this is a strain of LT22; it put out a phage (we didn't know much about phages back in those days either), a phage PLT22, which went across the filter, attacked LA 22, L stands for and A for auxotroph; auxotroph strain 2 makes P22 grow on that strain, produces a substance which we ultimately call filterable agents -- it's not very original but it passed a bacterial free filterable agent and it goes back and tranduces -- we didn't have the word transduction at that particular time -- but changed LA22 to make it look like a prototroph, even though it had some mutants originally. And that's what really happened in that U-tube. Lights please.

But that's not what we did and we understood it to do. Somehow or another we got very classical -- medi-classical -- in microbiology. For microbiologists were always finding strange creatures, things that passed filters, strange forms. And Josh gave the history of the theories of sex or non-sex in the growth of bacteria, but there's always on the side, strange creatures, bacteria that could change into each other. In fact, there was once upon a time a theory that all bacteria were one species and that they just continuously change A into B. Well they had something which they called "filterable forms" was studied by and Deanis. They were capable of passing sterile filters, came from most bacteria under certain specified noxious conditions. They were sort of a way for a bacterium that couldn't produce a spore to escape the sad and bad world that was attacking it. And they went through the filter and then they would grow on certain special medium and they could even resurrect themselves. And somehow or another, we came to the conclusion that the filterable agent was an L form. Even today I can't figure out if it were an L form, what it would have done to only have affected a single marker because (next slide), now this is just a titration of FA. It's grown in filterable agents; it's grown by mixing the two cultures together, killing off the bacteria and then you have

a solution that changes auxotrophic bacteria to prototrophic bacteria. Any auxotroph to any prototroph; any Lac - to Lac +; except it doesn't do it two at a time. (Next slide) There's no linkage. It's just like transformation actually. As we understand it, this is just a showing that FA is a filterable agent from a strain, +
which transform every one of those characters; but only one at a time. So what the FA (filterable agent) did was precisely what the crosses did. So we were studying the right thing and we were looking at it in the right way but we were calling it the wrong thing and we were really not doing the proper genetic analysis. (May I have the lights please).

But there are lots of ways of getting FA to appear and it was simply because we did not know that you can induce pro-phages by a variety of different agents. So you put something into a test tube, most of our salmonella cultures carried a pro-phage, you get some activity. And you say, my God, it's just the way and Nobel said you add a little of this and a little of that and the next thing you know is that you have filterable activity. It was all wrong. I don't understand it all. But I mean, how could we be so wrong. But we get even wronger.

In May of 1951 we write an overwhelming paper for the Cold Spring Harbor Symposium. This paper was delivered by Josh at the symposium. I don't remember if it were six or nine hours, at least. It's a summary of three years work in the laboratory. Everything was in the laboratory. It has four features -- formal genetics of coli pheno; genetics; bacterial psychology; extra-cellular genetics (that's Lander and things like that) and transduction. They were all in there. I'm particularly concerned about the transduction section. I would say that the data in that paper are all really quite good. I would say that almost every interpretation in that paper is wrong. Maybe everyone. I think the pheno-genetics are a little better than most because we ran into trans-effects; at least we stated them properly.

So I went to the Cold Spring Harbor Symposium. And for me that was a tremendous experience. I met all the famous geneticists and it was an interesting time. The opening talk was given by Goldschmidt, who said there was no such thing as a gene; and the closing talk was given by Sonnenmorn who defended who was a German scientist who had the most strangest results in segregation in . In between, Barbara McClintock gave her famous paper which she claims nobody understands. Compared to our paper, her paper was a masterpiece of lucidity. There is a great paper by Norman Horowitz, which really showed the

end of Tape # 1

Tape # 2
preparation of phage, by growing the phage on it. By this limited test, one can now presume three by repeating in all combinations,one can get a complete analysis of all nine probes. It's clear that this is an absolutely genetic phenomenon. And then there's one last thing that bothers me. It bothered us at that time. I guess, today, it would be taken for granted. Streptomycin resistance, which we know is a recessive mutation and it does transduce. Recessive can be transduced as well as the dominant. And all the while I'm doing these things, I'm slowly building up my arguments. It is a phage. (Next slide). (let's skip this slide; this is just a little linkage in transduction). (Next slide please).

And these are a lot of experiments which we all just pooled to show that because what we're doing here is looking for a correlate between the transducing activity and the phage activity. So we have the size, and its common by ultrafiltration and ultracentrifugation.

An antigen. An antiserum is prepared against the preparation and the both of them go off at the same kinetics. And that's highly unlikely that you're going to activate two antigens with the same kinetics.

The heat sensitivity. The kinetics and the activation. The site of absorption, that is, there are strange existed absorbed phage and strange existed absorbed FA; and they're always the same. And that's nice. Asthmatic shock, both in the resistance etc, etc, DNA, that was important. It wasn't just a piece of DNA sitting on something or not. No effect. Remember this is 1951. Well, we all knew about DNA and transformation in pneumococcus. We all were still just a little bit skeptical. The tryptasome effect in ultraviolet does separate .

Well, I've taken you now through almost to November, 1951. And by this time, it's clear it's a phage, and the name transduction is created. Transduce -- meaning -- to lead across. In a few weeks, Lander transduction, is discovered, but that's another story.

Thank you.