

This Week in Virology

With Vincent Racaniello, Ph.D.

Episode 197: Cloning HeLa cells with Professor Philip I. Marcus

Recorded 15 May 2012

Aired 26 August 2012

<http://www.twiv.tv/2012/08/26/twiv-197-cloning-hela-cells-with-professor-philip-i-marcus/>

Transcribed by Raphael D. Fernandez 9/11/2012

VINCENT RACANIELLO

For today's episode, I travelled to Storrs, Connecticut back in May of 2012 where I spoke with Professor Philip I. Marcus a distinguished professor there who spent his career working on viruses and interferon. Prof. Marcus began his scientific career in the early 1950s and if you recall that was about the time when HeLa cells were developed. And one of his projects was to try and figure out how to clone the cells. That is, how to make populations of cells derived from a single cell. And although we do this all the time nowadays, back then, it wasn't so easy. So listen to my conversation with Dr. Marcus as he recalls how he got the idea to clone HeLa cells and how he finally did it, the first person ever to clone HeLa cells.

I have your original letter that I first got in—it's dated February 5, 2010—that's when you wrote the authors of the textbook of virology. Do you remember that?

PHILIP MARCUS

Ah, yes. Oh, we're going to have a special session on that or are we going to settle that? We're supposed to make the correction.

VINCENT RACANIELLO

So we're working on the next edition now and I'm going to make sure it's fixed. Absolutely. And today, I'm going to...

PHILIP MARCUS

I will ask you to think about what I say and recognize Szilard's contribution was not "trivial" as Puck...

And when he was being interviewed by Bill Lanouette for this book which...

VINCENT RACANIELLO

"Genius in the Shadows." This is about Leo Szilard.

PHILIP MARCUS

And out of that whole book, there are two pages devoted to the biology that I'm going to talk about.

VINCENT RACANIELLO

Oh, I see your name and Puck's name, very good.

PHILIP MARCUS

Yes.

VINCENT RACANIELLO

All right.

PHILIP MARCUS

And Lanouette, who got great notices for the book, he really captured the guy, interviewed Puck and he got one version of cloning. He interviewed me and he got another. And so he went back and forth twice and checked with a lot of other people. Puck ended up saying that Szilard's contribution was "trivial." And I say it was "seminal." Fortunately, I think Lanouette listened to both and he came up with this version which is close to what I'll relate.

VINCENT RACANIELLO

Alright, ready to go? Okay. Well, we've already started.

I'm visiting today in Storrs, Connecticut with Prof. Philip Marcus who is a professor in the Dept. of Molecular and Cell Biology at the University of Connecticut. We've been planning to have a chat for quite a few years now.

PHILIP MARCUS

I think so.

VINCENT RACANIELLO

So thanks for having me and to talk about the early years, maybe of cell culture, if we could call it that.

PHILIP MARCUS

It was the early years as you can see.

VINCENT RACANIELLO

Before we start talking about science, tell us a little bit of your background. Where are you from originally?

PHILIP MARCUS

Springfield, Massachusetts.

VINCENT RACANIELLO

So you're a northeasterner?

PHILIP MARCUS

Absolutely.

VINCENT RACANIELLO

And you went to school in Springfield? Where did you go to college?

PHILIP MARCUS

Well, World War II was on then. And if you volunteer at 17, then they would put you in college, the government. And since neither of my parents got beyond the sixth grade, they really didn't know what college was. And they had saved up this magnificent fund of \$300.00 to put me through college. So I thought Uncle Sam might be the way to go and I signed up and where did they send me? To a place called "Storrs," the University of Connecticut, in 1947 I think, '45.

VINCENT RACANIELLO

It's where we are now, right, although it's probably very different back then.

PHILIP MARCUS

I'd say about 1/10th the size. It's amazing. But we went to school six days out of a week. What I thought was 24 hours a day. And at the end of that time, I had earned two years college credit; really the way to go. And that launched me into science. My parents thought I should be a violinist. And they spent—wasted money on that. They finally concluded after I had got some spring papers, frozen them into ice cube trays and then when my mother serves drinks...they would thaw out and the spring paper—that was it. She knew I was committed to science. And then after that, during the war, I went to school here. I came back. I went to UCLA for a semester then USC and then to the University of Chicago where I can pick up the story we're probably most interested in.

VINCENT RACANIELLO

Is that for PhD, the University of Chicago?

PHILIP MARCUS

No, I got a Masters there. I got Bachelors at Southern California.

VINCENT RACANIELLO

Okay.

PHILIP MARCUS

So here I am, a newly-minted microbiologist looking for a job at the University of Chicago. And I get a job in a laboratory that during World War II was devoted to the inactivation of bacteria in droplets of ethylene glycol. As fate would have it, the person who ran that laboratory during World War II was Ted Puck, my mentor-to-be. I didn't know him then. He wasn't there. He had gone to Colorado. I was introduced to this sterile room, maybe twice the size of the office here and I began acting as microbiologist. They would spray bacteria into the sterile chamber and spray ethylene glycol and the mixture at the right humidity would inactivate the bacteria. That was the thinking behind it.

So here I am on my new job. I walked in early in the morning. I'm a microbiologist. So I'm used to seeing small things. I looked into the sterile chamber and I say, "Is that a mouse?" "Yes, it's a mouse in the sterile chamber." So I dutifully reported this and that was a little disturbing but nonetheless there was a mouse in the sterile room. So they cleaned that up. All right, I reported it. I don't think they were too happy about it but I continued my work and one of the first things I did there—this is about 1953 now.

VINCENT RACANIELLO

Ah, the year I was born.

PHILIP MARCUS

Aha! I was to give a seminar. I thought, "Oh, that's exciting. You have a job. They pay you. You give a seminar." So I talked about what was, I thought, very exciting then. This new work of a fellow called Joshua Lederberg and bacterial conjugation and sex. I'm all excited. So I got a lead brick. Okay, no more seminars for me.

VINCENT RACANIELLO

This was the Department of Microbiology in Chicago or something else?

PHILIP MARCUS

No, it was a separate unit tied to this ethylene glycol. Okay. Then my next project was we've been spraying E. coli into the sterile room inactivating it and collecting survivors and growing them up and we keep doing this, so we're many cycles. And we found that we can convert a gram-negative bacterium into a gram-positive bacterium. Okay. And they were preparing a manuscript on this and they wanted me to see the slides. So I'm looking. They had all the slides lined up and, yes, we started with a nice pink E. coli and we kept going and then all of a sudden there was a rod, gram-stained rod and then more and more. Sure enough, there was a population of gram-positive rods. It didn't

take me long to figure out what was going on. What took time was how to tell them what I thought was going on. And as gently as I could, I said, “If you heat this preparation out here, you’ll find it survives under conditions where this won’t be.” And I told them these are bacilli and they have spores in. Okay. So, fine, I made my judgment. About three or four weeks later, my contract was terminated and that turned out to be a turning point in my life.

I had a friend at the University of Chicago who knew I was now looking for a position and she mentioned two people that were looking for a research technician: Aaron Novick and Leo Szilard. I have heard of Leo Szilard, the atomic physicist, but they had a laboratory on the other side of the campus in the Radiobiology Institute where Szilard had turned his work from physics and the atomic bomb and the Manhattan Project into biology, sort of a compensation for all the destruction that it cost.

VINCENT RACANIELLO

He became part of this phage school eventually, right? With Max—he’s a famous phage...

PHILIP MARCUS

Delbruck.

VINCENT RACANIELLO

Max Delbruck, right?

PHILIP MARCUS

Szilard spent time in Cold Spring Harbor amongst the...

VINCENT RACANIELLO

So Delbruck was also a physicist as well. So the biology began to attract physicists, right?

PHILIP MARCUS

Yes. That was the germ of molecular biology eventually. So they set up an interview with me with Aaron Novick and Leo Szilard. I meet them at the tennis court. “Oh, well, all right.” I read later from Lanouette that, for some reason, Szilard likes to sit by tennis courts. I met both of them. We had a conversation and Novick is looking at Szilard and Szilard is looking at Novick and after about 15 minutes, they hired me; changed my life entirely. These people did science at the highest level.

So I moved over to them and worked there until Novick went on a sabbatic and then I worked with Paul Talalay in the Ben May Cancer Lab at the University of Chicago; again, a fine group of people. They invited my former bosses to come over and discuss their experiment—changing E. coli. They didn’t bother to attend.

But during that period when I was with Szilard, there was a visit from a fellow named Ted Puck. He had established the Department of Biophysics at the University of Colorado Medical School in Denver. And he was looking for a research assistant. He liked to hire people out of Novick and Szilard's lab where they were doing mutation of bacteriophage in the chemostat.

So I spent a lunch with Ted Puck and he was telling me what he wanted to accomplish. He'd like to be able to take single mammalian cells and grow them with the ease that the bacteriologist did. You just plate them out. You get colonies. You can then measure the effects of radiation, antibiotics, whatever stress you want. But at the moment, that wasn't possible with mammalian cells.

VINCENT RACANIELLO

Can you remind us—so this is 1953?

PHILIP MARCUS

This is 1953, late.

VINCENT RACANIELLO

What is the state of mammalian cell culture at this point?

PHILIP MARCUS

Well, when I heard he was interested, that was pretty heady stuff for a new microbiologist. I said "yes" and I start reading up on the subject. So I read about a fellow named Albert Fisher dating back to '43. He had a concept that you won't be able to get mammalian cells to grow a single cell because they have evolved with neighbors. It made sense. So it's not going to happen.

I ended up in Puck's lab in late summer of '54. I go to the lab—it's in the basement, his office is upstairs—and he has a post-doctoral person there, Roshan Christensen. And she had capillary tubes all over the laboratory which had single cells in it. This was based on work that Wilton Earle and Sanford at NCI at the time. They were attempting to clone mammalian cells. Earle had the notion that the reason single cells won't grow in culture—and they didn't, you needed mass culture—was they were losing some diffusible element. He could get around it by diluting the cells, taking them up in capillaries where you could confirm you have one cell per capillary. And so he did this with hundreds of capillaries. Once the cell was inside, this would be immersed in a petri dish with growth medium and he was successful to the extent of maybe 1% to 4%.

VINCENT RACANIELLO

So how was success measured, by the cell multiplied...

PHILIP MARCUS

By the cell—a single cell, confirmed microscopically—multiplying to the point where it would grow out at the end of the capillary, into the plate and you now have a clone. So these were mouse L cells essentially.

VINCENT RACANIELLO

So was that the first continuous cell line?

PHILIP MARCUS

It would be the first continuous line.

VINCENT RACANIELLO

Who did that?

PHILIP MARCUS

Wilton Earle, Sanford and Likely, I think.

VINCENT RACANIELLO

And these are transformed cells, right?

PHILIP MARCUS

I'm not sure that they were.

VINCENT RACANIELLO

That they were...

PHILIP MARCUS

They were from mouse.

VINCENT RACANIELLO

They were immortal basically, right?

PHILIP MARCUS

Yes.

VINCENT RACANIELLO

Okay.

PHILIP MARCUS

The problem with that—that's the lab I walked into when I joined Puck—was 1% to 4% of all the capillaries. The awkwardness of the capillary, you can't do the kind of experiment you want. So the first thing I tackled was, "All right, get rid of the capillaries and let's go to microdrops." Same concept: small drop and you can take a Petri dish, put a dozen microdrops in it, confirm they have one cell and you can put them in with different amounts of cells as long as you put maybe 20 or more into a microdrop.

VINCENT RACANIELLO

Twenty [more] cells?

PHILIP MARCUS

These were HeLa cells.

VINCENT RACANIELLO

You switched to HeLa now.

PHILIP MARCUS

I didn't switch. HeLa cells were there when I came. I think Puck had gotten them from Tuskegee Institute at that time. They were there but they would only grow on mass culture and even the HeLa cell going down to the capillary did not do well.

VINCENT RACANIELLO

Let's explore that briefly. The HeLa cells made by George Gey in 1951, is that correct?

PHILIP MARCUS

That's about right.

VINCENT RACANIELLO

So you could only grow them in plates. You could trypsinize them and replat them but you couldn't do single cell?

PHILIP MARCUS

No, you have to keep the numbers up. And when we went to microdrops, the critical number was about 10 cells per drop. If you have that many in, they'd take off and the drop would be confluent. So...

VINCENT RACANIELLO

If you put one in...

PHILIP MARCUS

One to five, somewhere in there; 1% to 4%, just like Earle's work, so we were ahead. Fortunately for science, this was now late summer '54. There was a visitor to the laboratory by the name of Leo Szilard, the very person whose lab I worked at the University of Chicago. Unbeknownst at that time, he was negotiating a position with Puck in Colorado because his wife, Gertrud Weiss, had a job in a hospital in Colorado. So he came out and it seemed very natural to take me out for lunch and find out how things are doing. I hadn't been there very long but did the micro work. We're at lunch and I'm telling Szilard, "Well, you know, it's not going good enough to grow the single cells. That's still a problem." We chatted about this awhile and then Szilard became very quiet. I learned at Chicago, when he's quiet, you don't want to interrupt. So I didn't say a word. And then he finally says, "Well, you know, if you can grow mass cultures, the

secret is not to let the single cell know it's alone." And I'm thinking to myself, "Oh, atomic physicist now to the psychobiology of the single cell." Well, it turns out he really meant in a biochemical sense. And we spent the rest of the lunch working out a system where you could put a single cell in the environment of a mass of cells so that whatever this diffusible substance or substances or factors was, there'd be plenty of them. And I rued to this day, we drew out a schematic on a paper napkin. I don't have that. We worked out a system that should fit Szilard's requirement not to let a single cell know it was alone. I'm all excited about this. I've been there maybe with Puck only two or three months and lunch was over and I said, "Aha, go to see Puck in. Tell him about this exciting." So I go in and I see Puck and I tell him about this. And he's quiet. I said to myself, "Gee, maybe all great scientists are quiet. So I have to be quiet and listen to them." Finally he spoke and he said, "No, that's not going to work. Forget about it." I was absolutely crushed. I did forget about it, for about 20 to 30 seconds. That's how long it took me to leave his office. I got outside and I said, "No, this is too good." I said, "I'll try it. If it doesn't work, we'll know. If it works, I'll worry about it." I went to see the machinist in the Biophysics department, a fellow named Bob Edgerton. I showed him the schematic. Could he make this little plastic platform? He cut some out of plastic and I sterilized them with UV and I started an experiment on Friday. I put down a mass culture in a small Petri dish. I put down this platform, which in practical terms, was about 200 cell-width or diameters.

VINCENT RACANIELLO

What does this look like? Is it just a round cylinder sitting on the bottom?

PHILIP MARCUS

No. I have picture of it. You'll see later. It's sort of flat but it's raised above the monolayer. On that, I separately put microscope slides that have been cut—glass slides—about so big and inoculated many drops again with one cell or no cell, taking advantage of the Poisson distribution. Many of them were zeros but I could confirm microscopically they were one. They will settle down and attach in a different dish.

VINCENT RACANIELLO

These were HeLa cells?

PHILIP MARCUS

These are the HeLa cells. Once they're attached, they're very stable. Then I would take the cover slip, place them on this plastic platform, two of them, and then submerge them in the medium where the mass culture was.

VINCENT RACANIELLO

And the mass culture is HeLa cells also?

PHILIP MARCUS

Yes. Put the cover on. Put it away. I did this on a Friday. I came in Saturday. Every plate I had put one cell, there were two. Every single cell had doubled. “Oh, that’s nice.” I came in on Sunday and every plate that had two cells now had four cells. I said, “That’s even nicer.” I said, “It’s working.” So on Sunday, I called up Szilard and he came down to the lab with his wife, Gertrud. I showed him this. And he has this big grin on his face. He’s just a big person. He was satisfied right there. It worked. Okay? That’s it. Then he turned to me and says, “Well, you have to call Puck.” And I said, “Well, I wasn’t authorized to do the experiment.” So he said, “No, you have to call Puck.” Of course, he was right. I called Puck. He came down to the lab on Sunday and I told him the setup and he looked through the microscope. He saw these four-cell clone. He didn’t say a thing. He just disappeared. The one thing I cannot remember is how long I didn’t see him. Okay. It had to be days. His office was upstairs. The lab was downstairs. He was not a lab person. I think once in all the time I was there I asked for help.

VINCENT RACANIELLO

You were a research assistant at this time?

PHILIP MARCUS

I was a graduate student at the time going from Masters to PhD in Microbiology with—also in Biophysics. So I think it was a few days or a week then he came down to the laboratory as if nothing had happened and he says, “What we have to do is irradiate the bottom layer so that it will be biologically functional but sterile and then it can’t contaminate the single cell.” Not that it ever did in any of the experiments I did. That’s why I was making the point. The platform was in cell diameters, 200 cell diameters. So if a cell came off in mitosis, they go down by gravity. They’re not going up 200 cells...

VINCENT RACANIELLO

So it’s a raised platform for that...

PHILIP MARCUS

It’s a raised platform.

VINCENT RACANIELLO

Okay. Did you ever do controls where you just put a platform in without any cells?

PHILIP MARCUS

I didn’t but I saw no evidence of gaining any colonies. I would verify the microdrops.

VINCENT RACANIELLO

Yes. You start with one, the next day there’d be two and then four?

PHILIP MARCUS

Yes, I didn't pick up one layer on. And the physics, if I can use the term, was against it. So, great, and we went ahead and used the x-ray machine that Puck had bought for the department two or three years earlier and had never been used. We began to x-ray cells to sterilize them. And it worked. I'll show you some pictures later of the x-rayed giant cell. They produce large—they would undergo nuclear division but not cell division. So the cells got bigger and bigger but they were sterile.

VINCENT RACANIELLO

And the idea is they're producing...

PHILIP MARCUS

Whatever the nutrients were that the mass culture was there's every reason to feel that they were doing the same. It would take, after a dose of irradiation, you could plate them and they get larger and larger for about eight or nine days before they disintegrate. So at about this time, we used the x-rayed feeder layers and the platform that Szilard and I had suggested and that was the first publication at 1955.

VINCENT RACANIELLO

Who was on that paper? You...

PHILIP MARCUS

Puck and me.

VINCENT RACANIELLO

You and Puck. Okay. Not Szilard?

PHILIP MARCUS

Not Szilard. You have to remember I'm a graduate student now maybe only four or five months. Not quite used to how these things work. Besides, Puck had put a footnote in that paper stating Szilard's contribution. I'll discuss that later when we go over the slides because it's a critical statement but at the time if that's the way they do it, that's the way they do it. And so that paper—let's see, that's probably a good place to take a break because that paper came out, that was the paper that introduced the world to feeder cells. It was feeder cells that Szilard had actually recommended. And Puck had added the element of x-raying them so they wouldn't be able to divide and together that constituted the first paper.

VINCENT RACANIELLO

I'm looking it up at this moment. See if I can find it.

PHILIP MARCUS

I have given away all my reprints. I should have it right here to show you. I have it on this. And you will see it in the slide. It's the middle picture.

VINCENT RACANIELLO

Okay. So that's a diagram of this platform apparatus.

PHILIP MARCUS

That is the diagram and it was drawn by yours truly. As I looked back on it, my gosh, that's a lousy job. One part of the Petri dish is hanging over further than the other and it's a little crooked. But nonetheless, that's my figure.

VINCENT RACANIELLO

That's drawn with rulers and a pencil, right?

PHILIP MARCUS

Yes.

VINCENT RACANIELLO

I see. So then the cells are growing on this platform. That's great. And the paper described this...

PHILIP MARCUS

This procedure.

VINCENT RACANIELLO

Okay. Using HeLa cells, right?

PHILIP MARCUS

Using HeLa cells. Do you want to go to the figure six in that?

VINCENT RACANIELLO

Sure. Slide number six.

PHILIP MARCUS

It's the last page. In figure six, is the footnote that was in the paper, that 1955 paper, it says:

In our earliest experiments, test cells and feeder cells were placed in the same layer. We wished to thank Dr. Leo Szilard who suggested the more advantageous geometric arrangement in which these test cells were placed on top of a layer of the feeder cell."

That statement is not accurate. This was the first experiment we did. That was followed by the fact that we could plate the single HeLa cell in a layer of giant cells. There was enough room. They were settled in. And they would grow being fed by the giant cell. You didn't need the platform. But the first experiment was the platform as suggested by Szilard and this statement is inaccurate leading one to think that we had co-seeded the cells.

VINCENT RACANIELLO

So you didn't do that yourself? You never put the feeder in the test cells...

PHILIP MARCUS

Later.

VINCENT RACANIELLO

Later on. Yes.

PHILIP MARCUS

We use that procedure.

VINCENT RACANIELLO

And before you came to the lab, this hadn't been done?

PHILIP MARCUS

Prior to Szilard, none of this work had been done. In truth, I was the only one working on it. And I was in the lab and Puck never came down to the lab. And it was at that point that we did—I did cloning and developed these cloning cylinders—I have some out there, you can see them—and cloned the original HeLa when it was plated. You can see here. On this slide, it was quite heterogeneous. Page five.

VINCENT RACANIELLO

So these are clones of HeLa cells growing on a plate?

PHILIP MARCUS

That's correct. Because finally the media got better and we didn't even need...

VINCENT RACANIELLO

...the feeder layer any longer. How long did that take?

PHILIP MARCUS

Maybe a year or so, but feeder cells are still used to grow stem cells and fastidious cell. When you don't know why they're not growing, you just stick in the feeder. Well, here is the wild-type, uncloned HeLa. And then I picked the clone from then and you can see the uniformity picked up. I'll show you.

VINCENT RACANIELLO

So that is the first time that HeLas were cloned?

PHILIP MARCUS

Yes, using the cloning cylinders.

VINCENT RACANIELLO

So you would take a glass cylinder and place it on these colonies and remove some cells?

PHILIP MARCUS

Well, it was stainless steel but...

VINCENT RACANIELLO

Steel? Yes. We used to use glass and you would put a little trypsin in to remove the cells?

PHILIP MARCUS

You put the silicon on the bottom and you could press it right over the colony. That would stick. Remove the medium then add trypsin and let them come up and move them over.

VINCENT RACANIELLO

And these above are photographs of the colonies, the individual colonies of HeLa cells?

PHILIP MARCUS

Those happened not to be—these are different clones that came from the wild type. The ones in phase-contrast...

VINCENT RACANIELLO

...are not HeLa cells?

PHILIP MARCUS

...are probably not HeLa cells. My associate, Margaret Sekellick, reminds me they are probably WISH cells.

VINCENT RACANIELLO

So this cloning which was done was the first time a mammalian cell had been cloned and cultured? So it was done first for HeLa before the mouse L929 cell that you mentioned before? Is that right?

PHILIP MARCUS

No. The 929 cells were cloned in capillaries.

VINCENT RACANIELLO

Capillaries. Very inefficient, right?

PHILIP MARCUS

Yes.

VINCENT RACANIELLO

Okay.

PHILIP MARCUS

But they pre-dated this.

VINCENT RACANIELLO

Got that. And then eventually, you introduced your...

PHILIP MARCUS

We have that picture here.

VINCENT RACANIELLO

That's a picture of the capillary, right?

PHILIP MARCUS

Yes.

VINCENT RACANIELLO

That'd be interesting. You can see the cells growing out of the end of the capillary.

PHILIP MARCUS

They finally grow out. Here it is, single cell, they start, they take off.

VINCENT RACANIELLO

So I can put this in our video, right? That will be great.

PHILIP MARCUS

That is a duplicate of what—the only thing I left out is the cloning cylinder you'd take on back with this.

VINCENT RACANIELLO

Eventually, you adapted the HeLa procedure to these L cells or any other cells to get clones, right?

PHILIP MARCUS

Oh, once the HeLa...

VINCENT RACANIELLO

...then everyone else followed, right?

PHILIP MARCUS

Yes.

VINCENT RACANIELLO

And today cloning cells is very important. If you want to put a gene in the cell, you have to pick a clone with just that gene. It must remind you all the time of the work that you did, right?

PHILIP MARCUS

Yes.

VINCENT RACANIELLO

Is this a photo of you here, the first, the second slide?

PHILIP MARCUS

That's when I was at the University of Chicago working in Novick and Szilard's lab. So that takes us back to '53. Then I joined Puck. This is from Harris's book.

VINCENT RACANIELLO

Here's a photograph of Szilard and...

PHILIP MARCUS

...and his friend. When I worked in Szilard's lab, it was a little unusual. A primitive one might say. There was one phone. It was on the wall. When Szilard would come back from his trips around the world, fertilizing ideas to different scientists, he'd come back and he make all these phone calls on that one phone. I'm working in the laboratory and one day I hear him speaking to the University of Chicago operator. "Would you please get me Professor Albert Einstein at Princeton?" So I did hear one part of the conversation. It was in German. My German wasn't that good but I ended up at Einstein College of Medicine.

VINCENT RACANIELLO

So the science was very different back then? It was much less formal than it is now, right, the phone in the lab.

PHILIP MARCUS

Yes, that plus the fact if you had a respectable proposal, you could get it funded. And papers were written then—it was opened up with "So-and-so showed that this..." and then your work would follow. You show me a paper today that says, "So-and-so did this..." it's changed.

VINCENT RACANIELLO

Why do you think that's so?

PHILIP MARCUS

Because of the scarcity of money, the tight budgets.

VINCENT RACANIELLO

Yes. It's much more competitive now.

PHILIP MARCUS

Extremely so.

VINCENT RACANIELLO

You worked how long in Puck's lab?

PHILIP MARCUS

I worked from '54 to '60 then '60 I went to Albert Einstein College of Medicine.

VINCENT RACANIELLO

And that was as a faculty member there?

PHILIP MARCUS

As Assistant Professor and Szilard had sent a reference and he wanted to know why they didn't hire me as an Associate Professor. That's Szilard.

VINCENT RACANIELLO

Tell us, who was in Einstein at the time that you remember? Because I remember a lot of well-known virologists—Bernie Fields was at Einstein, right?

PHILIP MARCUS

Bernie Fields...

VINCENT RACANIELLO

Was he there at the time you went?

PHILIP MARCUS

I don't think so. Joklik was there.

VINCENT RACANIELLO

Bill Joklik. Now I know Don Summers and Ellie Ehrenfeld were there.

PHILIP MARCUS

They were there.

VINCENT RACANIELLO

The same time that you were there?

PHILIP MARCUS

Yes.

VINCENT RACANIELLO

And the DNA replication guy.

PHILIP MARCUS

Oh, yes. Oh, right.

VINCENT RACANIELLO

He is now—he may still be at Einstein actually, right?

PHILIP MARCUS

Is that right?

VINCENT RACANIELLO

I forgot his name.

PHILIP MARCUS

Oh, I know his name.

VINCENT RACANIELLO

You know who I'm talking about, right?

PHILIP MARCUS

Yes, great guy.

VINCENT RACANIELLO

I'm just terrible. I can't remember his name. I don't have internet or I would look it up.

PHILIP MARCUS

I haven't thought of his name in years and years.

VINCENT RACANIELLO

Yes. I think he might be in Sloane-Kettering now. No, probably still at Einstein but still doing DNA replication.

PHILIP MARCUS

No. The fellow—Jerry...

VINCENT RACANIELLO

Jerry Hurwitz.

PHILIP MARCUS

Hurwitz.

VINCENT RACANIELLO

Here we go. So he was there.

PHILIP MARCUS

And Philip White. They weren't virologists but they were top-notch biochemists.

VINCENT RACANIELLO

Okay. What did you work on in your first lab?

PHILIP MARCUS

I worked on a phenomenon—the first demonstration of the dynamic incorporation of a viral synthesized molecule in the cell, its transport to the cell surface and its movement

around the cell. You don't know about that paper? It came out in 1962. You were only so high then.

VINCENT RACANIELLO

I was 9 years old at the time. But this was influenza virus it sounds like?

PHILIP MARCUS

It was NDV and HeLa cell. I can show you some pictures later. But it turns out to be a classic because it showed how the hemagglutinin molecules first appeared at the polar ends of a cell if it was bipolar. And if it was tripolar, the red cells would stick on the tips. And then they would move in centripetally until the whole cell was heme absorption positive. After working that out I spent a year with Dulbecco at La Jolla and came back to Einstein and we were in the midst of a rubella epidemic. And I had a good colleague in paediatrics, Dave Carver, and he says, "You have to develop an assay for rubella virus. I don't have one. This is not a CPE. It doesn't destroy cells so you have to do something indirect." For some reason, I said, "Okay, let's see if rubella virus interferes with the replication of NDV." So we infect cells, Vero cells with rubella virus. You go back the next day, they're fine. Two day, three days, they're fine. No CPE. But if you challenge those cells with NDV, the high multiplicity, they're all infected. They were refractory. They would not absorb red blood cell. So we turned that into what is called a "hemadsorption-negative plaque assay." There are several viruses that are non-cytopathic that you can get plaques with that way. And someone went a little further—this is years ago—and he said, "While using this, we call it 'intrinsic interference.' The only cell that was blocked by NDV was the cell that had Rubella. Right next to it, no rubella, you got NDV to go." This fellow let the cells go and they killed all the cells around the original hemadsorption-negative plaques and you had a colony. You could tell it that way.

VINCENT RACANIELLO

So you were a few years—I wanted to bring up Dulbecco who, as you know, just passed away recently. He had spent some time...

PHILIP MARCUS

Yes. I spent a year there, great place.

VINCENT RACANIELLO

And he embraced cell culture very early on.

PHILIP MARCUS

I would say he probably was a pioneer in the sense with respect to virologists. I remember Puck coming back from a meeting at CalTech and he looked through a microscope and he says, "That's a metaphase cell." He got that from Dulbecco. That

was a heady time there because Puck had Sir Macfarlane Burnet before he was “Sir” and I met Jim Watson, Pauling, Dulbecco, just stellar people visited.

VINCENT RACANIELLO

Sure. So Dulbecco developed a plaque assay for animal viruses, of course?

PHILIP MARCUS

Yes.

VINCENT RACANIELLO

In the early '50s?

PHILIP MARCUS

Yes.

VINCENT RACANIELLO

I know that Howard Temin went to work with him and then used the cells to demonstrate transformation by RNA tumor viruses.

PHILIP MARCUS

When Dulbecco was plaquing polio on HeLa cells I think. Yes. And he would use neutral red to contrast it. And he decided, well, to get more contrast he painted the laboratory green. So when he held them up the red against the green gave you more contrast.

VINCENT RACANIELLO

Nice, very good. That was brilliant. That must have been sometime in the '50s, in the early days of cell culture combining it with virology. But, of course, in Puck's lab you weren't interested in virology. You were interested in cell culture, right?

PHILIP MARCUS

It's interesting. His work—I learned after the fact, he usually didn't discuss these things—was supported by the National Foundation for Polio. I was the first one who used the cloning and the virus combined. I had learned my plaquing from Seymour Levine. He was a post-doc at the time. And another interesting tidbit: I plaqued WEE on chicken cells.

VINCENT RACANIELLO

Western Equine Encephalitis.

PHILIP MARCUS

Yes.

VINCENT RACANIELLO

Okay.

PHILIP MARCUS

Which as you know is infectious for horses and people. Well, they didn't have a vaccine at the time for people. So guess what? We got the horse vaccine.

VINCENT RACANIELLO

Oh, that's something you wouldn't do today, right?

PHILIP MARCUS

I don't think you could do it today. Not knowingly anyway. So I get these beautiful plaques of WEE on chick cells, my first plaque experience. I got busy with another experiment. I added liquid neutral red to help visualize it. So I put the plaque plates back into the incubator thinking, "Oh, I want to see if they keep growing." I've forgotten about it. Came back two days later, took the plate out and had very unusual appearance. I can show you some pictures. The original plaques were there. They had not increased in size. They were surrounded by neutral red-stained cells. But between the plaque areas, all the cells were destroyed.

VINCENT RACANIELLO

Yes, interesting.

PHILIP MARCUS

After the fact, I realized I was working with interferon. I had taken this up to Puck and I said, "Look at this." He says, "Oh, we see this in phage all the time." That was that. But I never forgot that. I even took a picture of that plate and in 1962 when I was talking at Cold Spring Harbor, their symposia on the mobility of the hemagglutinin on the cell surface. I'm sitting next to a fellow who was going to give the lecture after me up front. We sat chatting and he's talking about this interferon. "What's an interferon? I never heard of interferon." It was Alec Isaacs, co-discoverer of interferon. I think it wasn't many years later that I introduced that in the Cold Spring Harbor course I taught with, oh, several people: Gordon Sato—who's this fellow from Rockefeller—Richard Franklin—you must know his work.

VINCENT RACANIELLO

Well, he was David Baltimore's PhD adviser at Rockefeller. He worked on mengovirus and then polio eventually. And that's where David started working on RNA polymerases. So I found the reference to your paper. Puck and Marcus 1955, "A Rapid Method for Viable Cell Titration and Clone Production with HeLa Cells in Tissue Culture: The use of X-irradiated cells to supply conditioning factors."

PHILIP MARCUS

Exactly.

VINCENT RACANIELLO

So I worked with HeLa cells all the time to study viruses and we use a clone called "S3."
So were these clones made using your technique?

PHILIP MARCUS

They were not only made using my technique, they were made by me.

VINCENT RACANIELLO

Really?

PHILIP MARCUS

Yes. So I, one day, clone three clones from the HeLa, from the wild type - 1, 2 and 3. Puck added the letter "S" which stands for Florence Sabin whose little laboratory was named after. It's quite a renowned...

VINCENT RACANIELLO

No relation to Albert Sabin?

PHILIP MARCUS

No relation to Albert Sabin. So S1, S2 and S3 and do you know S1 is still around?

VINCENT RACANIELLO

I didn't know that. No.

PHILIP MARCUS

S3 is the most popular. S2, you don't hear about because I caught a cold one day and I'm working with transferring S2 and it disappeared. That's the story of S2. S1 turned out to be deficient in the amount of inositol it put out and require. And so if you plated it, the plating efficiency was may be only 10% or 20% as opposed to S3 which was 100%. So that's why you don't hear much about S1.

VINCENT RACANIELLO

Well, we use S3. I have used them since 1979 and I never knew who cloned them and now it's really nice to know that you did that years ago and you probably know that many, many people use these cells in many different laboratories.

PHILIP MARCUS

I gather if you color them all up.

VINCENT RACANIELLO

That's quite remarkable. I like that.

PHILIP MARCUS

It was a remarkable cell in that it grew like wild fire. If the cell happened to be located near the edge of a plate, it would form a colony and it start—completed on the side of the plate.

VINCENT RACANIELLO

Right. Do we understand why HeLa cells grow so well?

PHILIP MARCUS

Probably because its chromosome composition is so complex and so multi it's just pouring out everything.

VINCENT RACANIELLO

Because no other cell line approaches it really, right, or is that not true?

PHILIP MARCUS

I'm not sure that that's the case. Vero cells do very well. We did the cloning with influenza virus that John Ngunjiri did using Vero cells. We hadn't been using them for cloning and the plating efficiency was low. I told him just keep rapidly passing them and you select four and now we have a line that clone as well.

VINCENT RACANIELLO

I want to ask you one more thing and you can—if you want we won't talk about this and we can delete—but as you know Puck wrote an article saying that your version of this story isn't...

PHILIP MARCUS

I'm aware.

VINCENT RACANIELLO

Correct. So would you rather not discuss it or...

PHILIP MARCUS

He wrote the article. He was interviewed by Bill Lanouette.

VINCENT RACANIELLO

This is for this book on Leo Szilard?

PHILIP MARCUS

For that book.

VINCENT RACANIELLO

Bill Lanouette "Genius in the Shadows."

PHILIP MARCUS

Right. Lanouette tells me out of all this book, the file on these two pages is thicker than any other file he's gotten, trying to get the story straight. And the reason I don't hesitate to comment on it is it took me 50-plus years to figure out what footnote six meant. What it meant was: we did this before by coplating and then Szilard offered this platform. That isn't the way it happened. And I know because I did both. I was the only one working in the lab on that. And there's another clue as to why his version isn't correct. He wrote a small monograph called "The Mammalian Cell as Microorganism." When I was there, he mentioned that he was writing a book and he couldn't think of a title. So I was reading something about the clones from Macfarlane Burnet's book. So I gave him this title. That's fine. Okay. Puck wrote the book which is unique in that there's no attribution in it to any of the figures that are there. In my naïveté as a graduate student I'd find something going on in the lab that was fascinating. We had an incubator with an iron grid where you put the bottles or glass plates. And I'm sorry I don't have his book here. When I looked at the cells plated, they followed the iron grid pattern showing how sensitive they were to heat. In-between where it was air, they weren't growing. I took this up and incorporated and most all of the early material I had supplied. But the thing that amazes me about that book and I've been over it twice, it does not quote the original '55 paper in the opening chapters. It says "selected references" and the selection was against the original paper. Now why would someone do that?

VINCENT RACANIELLO

We'll never know.

PHILIP MARCUS

We will never know but footnote six is there.

VINCENT RACANIELLO

Right. And Dr. Puck has passed away. He seemed to be offended by Szilard interfering in his laboratory.

PHILIP MARCUS

Yes. And I am so sorry about that. As I said, I did not know at the time they were negotiating. However, Puck accused Szilard of being an interloper, interacting with the students. I had worked with Szilard. He was visiting. He was a few doors down from me. Nothing could have been more natural to have that meeting. Now, over the years, I don't have to tell you someone comes up to you as a mentor and presents you with a solution to a problem that you spent a lot of time on, how big a person you are determines how you receive that information.

VINCENT RACANIELLO

Of course.

PHILIP MARCUS

And to Lanouette's credit, apparently in a letter that Puck wrote to Szilard saying he's sorry there wouldn't be a position there. I can probably find it later. It's quoted here. "But your brain is so much stronger than mine that I don't think we would work together." And that's in the book.

VINCENT RACANIELLO

It seems to me that you would want someone as brilliant as Szilard to be helping you, right?

PHILIP MARCUS

I would say every time there's an opening in the department, I said, "Go outside and get the best person you can."

VINCENT RACANIELLO

But Szilard has no ulterior motive for helping you other than he was interested, right?

PHILIP MARCUS

Absolutely not. No. He got his delight came from seeing it worked and that's it.

VINCENT RACANIELLO

Right.

PHILIP MARCUS

And he did this throughout the world he would visit. Monod's laboratory, he was behind some of the basic thinking there.

VINCENT RACANIELLO

Truly altruistic, right?

PHILIP MARCUS

Yes.

VINCENT RACANIELLO

Wonderful story.

PHILIP MARCUS

A great person.

VINCENT RACANIELLO

I know that we have the attribution of cloning HeLa cells wrong in our textbook, so we will fix that in the next edition.

PHILIP MARCUS

I think you recorded in saying that.

VINCENT RACANIELLO

That's fine. No problem. Because I'm one of the co-authors, as you know, I'll make sure it's fixed.

PHILIP MARCUS

We used your textbook as you know. I send you whatever—it's sitting up there next to...

VINCENT RACANIELLO

I see it, several editions. And you teach a virology course here to undergraduates, is that right?

PHILIP MARCUS

Yes.

VINCENT RACANIELLO

Do you talk about these early days of cloning?

PHILIP MARCUS

I do.

VINCENT RACANIELLO

Did they appreciate it? Do you think?

PHILIP MARCUS

I actually asked them if they do. And some of them do. It's a little heady for them but lately I have been speaking to a colleague's class in ethics and so I've gotten together these slides. In the last day of the semester I spoke to his class and covered much of the material we're discussing now. When I was finished, the class applauded. That's a rare event in most classes. So I think they got the picture because it's become clearer and clearer to me as I think back on it and the evidence: no quotation of the original paper in the book; the footnote which is not accurate; trivial contribution, there it is in the first paper, the platform.

VINCENT RACANIELLO

So you said you record your lectures?

PHILIP MARCUS

Yes.

VINCENT RACANIELLO

Are they online or they're just for you?

PHILIP MARCUS

No, I have them all on disk. Margaret Sekellick sends them over to the UConn Husky site. Everyone who signed up for the course has access to them. She does slides, every slide I go up as well.

VINCENT RACANIELLO

Wonderful, great resource.

PHILIP MARCUS

It is. They like it. They love it. And it's not satisfying. Now they wanted streaming. So when they're walking along, they can listen...

VINCENT RACANIELLO

Of course.

PHILIP MARCUS

Yes, of course.

VINCENT RACANIELLO

That's technology.

PHILIP MARCUS

But you've come to the ultimate of technology.

VINCENT RACANIELLO

Well, I put my lectures online. Anyone can access them. This semester, I just finished teaching my class. We had 14,000 people subscribing to my course which I think is amazing.

PHILIP MARCUS

Fantastic. I thought I had set aside—well, I'll find it. I have one of the lectures that describe the acquisition of new viral molecules on the cell surface. I meant to give that to you.

VINCENT RACANIELLO

Well, I want to thank you for taking time to tell the story today. I appreciate it. And I think it's good for all of our listeners to hear it from someone who's there because there aren't many people who lived through the very early day of cell culture and virology. So it's good to hear the story.

PHILIP MARCUS

It was rather primitive. This picture you see here. These are the glass plates. There were five colonies placed. Single cells placed here. Five times more placed on this one. So there were 25. And this is a picture of one of the clones. And we didn't have any equipment to take pictures of clones apparently. So Puck goes and finds this

commercial ad and this fellow comes with a bellow that long and he sets it up on a tripod. The bellow is way out here and he got his film there and this is the result.

VINCENT RACANIELLO

Wow, yes. I was going to say you probably couldn't photograph the results very readily. But nowadays, of course, it's easy. How things change, right? All right, thanks so much.

END OF INTERVIEW

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Transcribed by Raphael Fernandez of *The Learning Blog* (<http://raphaelfernandez.com/>).