Philip I. Marcus: A Gentleman of Science (1927–2013)

Margaret J. Sekellick

The Early Years

PHILIP IRVING MARCUS WAS BORN in Springfield, Massachusetts, on June 3, 1927. He lived with his parents and sister in a house not far from Forest Park, a large, beautifully kept park in Springfield. This park was his backyard, so to speak, and he would go there often, year round, and explore the woods and ponds and streams and use his imagination to play the kind of games that children play. Philip loved to tell the story of how, when he was about twelve, he decided to collect some spring peepers from one of the ponds in the park and freeze them in an ice cube tray, one peeper per cube. His stated purpose was to see if they would survive being frozen and then thawed. He placed the tray into the freezer in the family's kitchen. It's not clear if he knew his mother was going to be hosting her lady friends for an upcoming afternoon of cards and that the ice cubes would be used to keep their lemonade cold. In any event, the experiment came to an abrupt end when the ice cubes began to thaw, and the spring peepers jumped out of the drinking glasses of his mother's friends. Philip never commented on whether he suffered any repercussions for his "experiment," but he did say that this was the point at which his parents recognized his love for science and ended their dream of him becoming a violinist, something to which he did not object (Marcus 2007).

Philip went on to attend Springfield Technical High School, which was the STEM program of its time, emphasizing science and mathematics in the curriculum. He was an all around good student, but he especially loved science and math courses. For fun, he joined the track team and specialized in the 100-yard dash. Apparently he was pretty good, as his team won a number of meets and were the Western Massachusetts and Interschool Track Champions in 1944. Philip boasted that his best time was 10.3 seconds, only 1 second from the world record at that time. When I asked him how much he practiced he said his secret to winning was to "not" practice. Practicing tired him out, so he saved it all up for the meet and then ran for his life. During the summers he worked at various jobs, including a stint at the Springfield Forge testing metal ingots destined for the manufacture of rifles. This was wartime, money was tight, and everyone who was capable of working had a job.

Philip knew he would have to go to college to pursue the kind of career he dreamed about, but money was tight and his

parents could not afford the cost. His decision to join the Air Force upon graduating from high school (in 1945) was intended to help him achieve his educational goals. He qualified for the Army Specialized Training Reserve Program (AF-ASTREP), which was an accelerated program that would provide the equivalent of 2 years of college credit after only 1 year of classes. He spent 6 months at the University of Connecticut in Storrs, and then

another 6 months at the University of Maine in Orono. During this time, he spent 8 hours a day in class, 6 days a week. Evenings were for studying and sleeping. Time for sleeping was at a premium because failing his studies was not an option. He recounted how he and his classmates had to choose between sleeping an extra 30 minutes in the morning or going to breakfast. That was a difficult choice for Philip because he liked to sleep and he loved to eat, but I think sleeping won out.

The College Years: From Baccalaureate to PhD

When he returned from active duty in 1948, after having served in a unit that supervised a German prisoner of war camp as part of the occupation forces in Istre, France, he moved to southern California where his mother was living and, using the GI Bill, continued his college education, graduating from the University of Southern California in 1950 with a BS degree in bacteriology. His decision to major in bacteriology was largely influenced by his reading of Paul DeKruif's *Microbe Hunters* and Sinclair Lewis's *Arrowsmith*. He then moved to the University of Chicago where he received an MS degree in microbiology in 1953. During his time in Chicago, he worked with Aaron Novick and Leo Szilard (nuclear physicist) and later with Paul Talalay (Marcus 2007).

He moved again in 1954, this time to the University of Colorado Medical Center in Denver, to pursue a PhD in

microbiology/biophysics in the laboratory of Theodore T. Puck. Philip later documented (Marcus 2007) that a luncheon conversation with Leo Szilard, who was visiting Puck's laboratory at the time, led to the concept and physical setup for the first clonogenic assay and feeder cell system, which made it possible to grow clones from single mammalian cells. This procedure resulted in the cloning of the immortalized HeLa cells, cells that are still used today in laboratories worldwide. It also led to the first determination of human cell sensitivity to X-rays and was responsible for the removal of hazardous X-ray devices that were used in shoe stores around the world (Marcus 2007). After receiving his PhD in 1957, Philip stayed on for several more years as an instructor and then assistant professor to develop procedures that allowed him to quantify the killing of single virus-infected cells, procedures that were fundamental to much of his future research (particularly once he returned to Connecticut in 1969).

In 1960, Philip moved to New York and spent 9 years on the faculty of the Albert Einstein College of Medicine in the Bronx, supported by a 10-year U.S. Public Health Service Research Career Development award. While there, he demonstrated the dynamic movement of virus molecules on the surface of infected cells and, with pediatrician colleague Dr. David Carver, developed a new test to detect rubella virus. During his time in New York, Philip was director for 9 years (1961–1970) of the post-doctoral course on quantitative animal virology and cell culture that was taught in the summer at Cold Spring Harbor Laboratory. At the time, this course was considered by many established scientists to be an important stepping stone into the exciting world of animal virology.

The University of Connecticut Years

In 1969, Philip returned to the University of Connecticut (UConn) where he was first appointed head of the microbiology section in the Biological Sciences Group. He spent the remainder of his career at UConn, where he created a Virus and Interferon Research Laboratory that was recognized internationally for its innovative studies, published over 130 scientific papers, and was awarded four U.S. patents. Other accomplishments included administration of the first Program Project on campus supported by the National Institute of Health and chairing the first biosafety committee.

As an early director of the Biotechnology Center, he helped attract companies with a focus on biotechnology to Connecticut. Then, for 12 years as director of the Biotechnology/Bioservices Center, he expanded the acquisition of state-of-the-art instruments, which drew scientists all across campus to the facility. He continued to advance biotechnology in Connecticut as a charter member of the Connecticut Academy of Science and Engineering and was pleased to learn that the Research Park, planned for Storrs, Connecticut, would be realized in the future. In 1987 he received the University of Connecticut Alumni Association Award for Excellence in Research and in 2003 was recognized as a Board of Trustees Distinguished Professor—awards that he cherished greatly.

His annual combined undergraduate/graduate course in virology, taught for 36 years, was highly rated by students looking to be challenged. Even though he had taught this course for many years, he still spent many hours every year scouring the current literature on the topics he covered in the

course so that he could present the most up-to-date lectures possible. He was always looking for new ways to present complex material in a clear and interesting way. About 15 years ago he announced to a class at the start of the semester that he was establishing the “Blue Lab Coat Award,” which would be given to the undergraduate student who achieved the highest grade point average at the end of the semester. The award came with the promise of a glowing letter of recommendation from Philip and a blue lab coat of their very own. A chance to be named the Blue Lab Coat awardee turned out to be a terrific motivating factor for many of the students. The blue lab coat was particularly special because for years Philip had been in the habit of donning a blue lab coat every day when he came to work. For him, this was an article of clothing that distinguished him from everyone else. If he actually needed to wear a lab coat to work in the lab, he would exchange the blue lab coat for a white tyvek disposable lab coat and then remove the disposable coat and replace it with his blue one when he returned to his office. This blue lab coat was his uniform. He wore it everywhere, to meetings, to lectures, and to class. Over the years, Philip received many accolades from his students expressing their appreciation for the opportunity to experience the challenge that taking this virology course offered.

Philip was a member and active participant in a number of professional scientific societies, including the American Society for Virology and the International Society for Interferon and Cytokine Research (recently renamed the International Cytokine and Interferon Society), which in 2005 named him as an honorary member and, in 2011, presented him with a Distinguished Service Award. Philip loved to attend scientific meetings as a means to stay current with the advances in virology and the interferon field as well as reconnecting with his many scientific friends, colleagues, and former students. He was especially fond of poster sessions during which he could be found strolling up and down the aisles engaging friends and strangers alike in discussions about their work. If one of his students was presenting a poster, Philip would ferret out his friends and lead them to his student's poster as a way of putting the student to the test—much like an oral exam but with a more friendly and relaxed approach. Most students found the experience of these encounters exhilarating and confidence building, but also a bit daunting, at least at first.

The JIR/JICR Years

Philip took over as editor-in-chief of the *Journal of Interferon Research* in 1984, following the resignation of Bill Stewart, the founder of the journal and society. This was a contentious time for the journal and Philip had a decidedly calming influence. One of his very good friends described him as follows: “He was always pleasant—even in the most trying of circumstances. His expression of calm during the most heated discussions with colleagues was something that I particularly wished that I could have embodied” (Anonymous). Another colleague described his style as “quietly competent” (Anonymous).

Philip was always looking for new ways to entice colleagues to publish in the journal. One year he had business cards printed up with a miniature JICR cover on one side of the card. This was long before cards like this were easily and quickly procured from internet vendors specializing in such

things. Anyhow, he brought the cards with him to the annual meeting of the International Society for Interferon and Cytokine Research (ISICR) meeting and handed them out to everyone he spoke to about submitting their manuscript to JICR. It seemed like people were lining up to talk to him and obtain one of these unique cards. By the end of the meeting he had given away all of the cards he brought with him. But his efforts didn't stop there. He was always trying to cajole editorial board members to publish in the journal, reasoning that if they, "the leaders in the field," published in the journal, then others would follow suit to be in their esteemed company.

Philip was extremely thankful for and well aware of the hard work and dedication of the many individuals who strove to make the journal a success. He expressed his thanks in an editorial that he wrote for JICR in 2002, when he finally stepped down as editor-in-chief (Marcus 2001).

Encounters with Double-Stranded RNA

I joined Philip's laboratory in 1969 at the same time he arrived at UConn. I had no experience in virology but was eager to enter the field. On Philip's advice, I had taken the Cold Spring Harbor Animal Virology and Cell Culture course in the summer of 1969 so that we could "talk the same language." In the laboratory at UConn, I recall hearing him talk excitedly about double-stranded RNA (dsRNA), both synthetic and viral, being found to induce interferon in chicken embryo cells. This was the work of Clarence "Bud" Colby and colleagues, which had been published earlier that year (Colby and Chamberlain 1969; Colby and Duesberg 1969). Bud came to UConn about a year after Philip arrived, and double-stranded RNA and what it might be doing in cells and to cells was a frequent topic of discussion. Philip loved theorizing about what might be going on in a virus-infected cell and how dsRNA might be involved. He literally could talk for hours and outlast just about everyone. He had chalkboards installed in almost every nook and cranny in the laboratory as a means of encouraging discussions. If he got involved in a discussion with someone and there was a chalkboard handy (this was long before the invention of the whiteboard), chalk dust would start flying around and end up everywhere, as diagrams of pathways outside and inside the cell would be drawn and modified over and over again and experiments proposed and planned and modified over and over again. One of the results of these early discussions was that we confirmed and extended the results reported by Colby and Chamberlin (1969) by showing that poly rI:poly rC complexed with DEAE-dextran, when added to primary chicken embryo cells aged *in vitro*, did induce interferon (albeit at very low levels) and was a very good inducer of an antiviral state in these same cells. For reasons I can't recall, we didn't publish these results until many years later (Sekellick and Marcus 1986).

It wasn't until 1975 when Philip and I were trying to determine the nature of the "cell-killing factor" produced by vesicular stomatitis virus (VSV) that we revisited the role that dsRNA might play in virus-infected cells. We had done experiments to determine which VSV gene(s) was responsible for killing cells and also to determine the nature of the cell-killing factor. We had hoped that the results of our experiments would lead to the identity of this factor; that was not the case however. This led to a number of lengthy

discussions during which we speculated on the nature of this hypothetical "cell-killing factor." For a number of reasons, dsRNA was an attractive candidate (see Marcus 1983 for an in-depth description of this subject).

In late April 1975, Philip met Robert "Bob" Lazzarini during the annual meeting of the American Society for Microbiology in New York City. Bob was aware of our cell-killing work and was interested in whether an unusual defective-interfering particle produced by VSV Indiana (MS strain) that had been isolated in his laboratory and identified as [$\pm$]DI-011 could kill cells. When the RNA of this particle was isolated, it was found to form a perfect hairpin structure of double-stranded RNA. They questioned if it could do this within the infected cell. Later that summer, Bob and Philip spoke again, this time by phone, and Bob agreed to send us some [$\pm$]DI-011 to test. The package with the [$\pm$]DI-011 arrived in late July and, thanks to Bob's generosity, we were able to do quite a few experiments with the material he sent. However, much to our great disappointment, [$\pm$]DI-011 did not kill VERO, a line of African green monkey kidney cells. This led us to look for a way to demonstrate that [$\pm$]DI-011 did, or did not, form dsRNA inside the cell. To do this, we chose to test whether or not [$\pm$]DI-011 could induce interferon in a cell system that was exquisitely sensitive to dsRNA, that is, aged primary chicken embryo cells.

When I got the results from the first test on interferon induction by [$\pm$]DI-011 in these cells, I went straight to Phil's office to show him the data. He tried to be calm about it, but I could see that he was as excited as I was. The defective particle turned out to be an excellent inducer of interferon, but interestingly, induction was extremely dependent on the multiplicity of the defective particle. Even more unusual, increasing the multiplicity of the [$\pm$]DI-011 resulted in a dramatic and almost complete shutoff of interferon (IFN) production. We didn't know what this meant, but we both agreed that we needed to do more tests to confirm this unexpected but exciting result. In the end, and after many experiments, we were able to show that the threshold for interferon induction by [$\pm$]DI-011 in primary chicken embryo cells was one [$\pm$]DI-011 particle containing one molecule of dsRNA per cell. Two or more particles in a single cell resulted in an inhibition of interferon production (Marcus and Sekellick 1977).

This wasn't the end to our fascination with double-stranded RNA. Indeed, if you were to look, you would find that the subject of double-stranded RNA is a thread that runs through almost all of our published work—right up to the present day.

Summary

Philip never tired of thinking, talking, and writing about dsRNA's roll in regulating cellular and viral activities and putting his ideas out there to stimulate the thoughts of others (Marcus 1983). The articles in this special issue are a testament to the many colleagues that have been influenced by his work and shared his fascination with this molecule.

A description of Philip's many scientific accomplishments goes beyond the scope of this piece. Suffice it to say that his many contributions to the fields of virology, cell biology, and the interferon system include the following: development of procedures to clone single cells (the clonogenic assay); demonstration of the sensitivity of cells

in vitro to X-irradiation; studies that defined the effect of aging cells *in vitro* and the demonstration that the *in ovo* development of the interferon system continues on time *in vitro*; the cloning of the first avian interferon gene; the discovery of intrinsic interference (a non-interferon-mediated viral interference) and development of the hemadsorption negative (HAD⁻) plaque test for rubella virus; studies describing transcription inhibition of viruses as a mechanism of interferon action; the discovery that one molecule of dsRNA was the threshold for interferon induction; the description and analysis of interferon dose (multiplicity)-response (interferon yield) curves; the discovery of interferon-induction suppressing particles and a description and analysis of their action in virus-infected cells; a description and quantitation of cell-killing particles; studies on the role of the interferon system in persistent infection; a demonstration of viral interference by defective-interfering particles in individual cells *in vitro*; the use of interferon induction as a quasispecies marker for VSV; the finding that influenza virus can express a transient resistance to interferon action; demonstration that oral delivery of interferon in chickens can inhibit viral pathogenesis; demonstration that oral delivery of interferon in chickens had an adjuvant effect on antibody production in virus-infected chickens; and the discovery and description of a double-stranded ribonuclease co-induced and secreted along with avian interferons (Marcus 2007).

Philip left a decidedly positive impression that went beyond his scientific accomplishments on almost everyone he met. One of his colleagues described him by saying "He...was generous in his comments and advice, always enthused, gracious, and critical when appropriate. Someone you could trust. We need more like Philip, one of the few who make a difference. He will be missed more than people realize, and a vast number will never know their loss" (Anonymous).

Another colleague summed it up by saying "Phil was not one to put himself center stage or trumpet his achievements.

It was his humility, his commitment to supporting others, his enthusiasm for science that set Phil apart. In every regard, he was a gentleman of science" (Anonymous).

Philip died at age 86 on September 1, 2013. He is dearly missed and will be forever remembered.

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Address correspondence to:
Dr. Margaret J. Sekellick
Department of Molecular & Cell Biology
University of Connecticut
91 North Eagleville Road
Storrs, CT 06268

E-mail: margaret.sekellick@uconn.edu