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IN MEMORIAM

Vincent Cristofalo (1933-2006): Extraordinary Gerontologist

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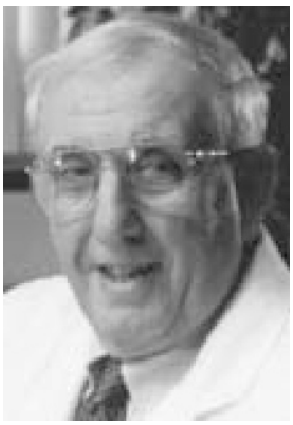

In this issue of Aging Clinical and Experimental Research, I have pleasure in presenting the proceedings of a symposium held during the annual meeting of the Gerontological Society of America, to honor the memory of Vincent Cristofalo (1933-2006). In his role as Associate Editor of Aging since 1989, Dr. Cristofalo's enthusiasm and dedication toward editorial activities were unlimited, and he was instrumental in raising the journal to ever higher levels, year after year. Since its initial publication, Aging has seen a marked rise in the number of submissions and published papers and an improvement in the quality and variety of published papers. Dr. Cristofalo's death is a loss for both research and publishing communities, but he has left an inheritance of rigorous scientific achievement and advance toward interdisciplinary and multidisciplinary work, and has contributed toward creating a journal worthy of meeting the challenges of future research on aging.

Gaetano Crepaldi
Editor-in-Chief

The Gerontological Society of America chose to dedicate a symposium at its annual meeting to honor the memory of Vincent Cristofalo, a distinguished biological gerontologist and ardent supporter of the society. The formal speakers in the symposium, which took place on Saturday November 18, 2006 in Dallas, were: Richard Adelman and Edward Masoro, two longtime colleagues; Leonard Hayflick, a mentor of his postdoctoral training; and Christian Sell, one of the many scientists, whom Vince had mentored. In addition, many in the audience recounted their special interactions with Vince. The following is a written version of the remarks of the formal speakers.

Richard Adelman

I was privileged to know Vince Cristofalo for almost half of a century as a professional colleague and friend. My brief contribution to this celebration of his splendid career is divided into two parts. The first describes how we met and interacted with one another. The second reveals a few of our more intimate moments together.

Vince and I initially met one another in 1962. This was prior to the decisions by either of us to opt for a career path in gerontology. The meeting place was the laboratory of one of the grand old men of cancer research, Sidney Weinhouse, who at the time was the Director of the Fels Research Institute at Temple University in Philadelphia.

Sidney's laboratory focused on the biochemical and hormonal regulation of carbohydrate and lipid metabolism in normal and cancerous tissues of rats and pigeons. I still vividly recall the gruesome scene on the day when Vince and I first met. A decapitated pigeon was flying around aimlessly and bouncing off the walls of the laboratory, splashing blood everywhere, until it finally crashed onto the floor. The student who had guillotined the pigeon in an effort to drain most of its blood supply prior to its dissection somehow had failed to grasp the bird firmly at the time of the beheading. A horrified Vince Cristofalo at that

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moment was the senior postdoctoral research fellow among a large group of trainees, and was acting as the tour director for my initial visit to the laboratory in which I was about to begin my graduate research.

At nearly the same time as this gruesome beheading, Vince also experienced the first major scientific confrontation of his career. Across town in Philadelphia at the Wistar Institute on the campus of the University of Pennsylvania, Leonard Hayflick's initial report of the finite *in vitro* lifespan of cultured normal human fibroblasts (1) was provoking great excitement and controversy. Vince was extremely impressed with the potential opportunity to study the biochemistry of cancer using the cultured cells as a model system. Sidney Weinhouse attempted to persuade Vince that there was no future in tissue culture; one of Sidney's rare errors in judgment. However, Vince, to his credit, persevered, stood his ground in this scientific disagreement with his mentor, and soon accepted an offer to join Len at Wistar. Parenthetically, to this very day I wonder whether Len is aware that Vince's decision to relocate to Wistar reflects his distaste for flying, bloody, headless pigeons as much as his appreciation for the ingenious studies of cultured cells.

Approximately seven years passed until Vince and I once again crossed paths in a scientific context in 1969, this time with both of us as committed gerontologists. I had completed my pre- and postdoctoral training, and then returned to Philadelphia to accept a faculty appointment at the Fels Institute. By that time, Vince already had begun to establish a biochemical context for understanding the nature of the finite proliferative capacity of cultured normal human fibroblasts as a part of Len's broader program at the Wistar Institute. He then inherited Len's gerontological presence at Wistar as Len relocated to Stanford University. Of course, soon thereafter, Vince achieved his own lasting status as one of gerontology's scientific giants.

Over the next 13 years, Vince and I, along with Ed Masoro, Jay Roberts, and George Baker, launched what was to become known in gerontological circles as the "Philadelphia Mafia". The five of us respectively held faculty appointments at each of five different academic institutions in Philadelphia; Vince at the University of Pennsylvania and Wistar Institute, Ed and Jay at what was then known as the Woman's Medical College of Pennsylvania, George at Drexel University, and me at Temple University. We literally behaved as if we were departmental colleagues at a single institution. We frequently shared each other's grants. We just as frequently competed with one another for some of those grants. We trained each other's students. We co-authored or co-edited a couple of dozen publications. We rigorously critiqued each other's ideas and data. As you might expect, we even provoked occasional administrative nightmares among our respective institutional bureaucrats across Philadelphia and within the financial offices of the NIH.

Eventually, two of us relocated beyond the Philadelphia area: first Ed Masoro to San Antonio, and then me to Ann Arbor. However, the geographic distance never was able to separate us. Over the past twenty-some-odd years, we continued to nurture, collaborate, and compete with one another, as well as to challenge each other's ideas and data and programs, whether at the request of each other or by invitation from various funding agencies and editorial boards. Our ability to launch and maintain this unique relationship over the years and across geographic and institutional boundaries, in large part was attributable to Vince's willingness to assign higher value to good science than to administrative ease, tradition, and sometime even common sense.

Vince and I shared numerous intimate moments that embrace a broad range of circumstances. We supported and encouraged one another during times of personal and professional crises. We traveled the world together to participate in various gerontological gatherings while our respective wives prepared back home to deliver daughter after daughter after daughter after daughter, and so on. However, I'll limit my specific reminiscences of this type herein to each of three specific incidents that for me typify Vince's love of science.

The first of these incidents actually has repeated itself frequently over the years, and consists of Vince's written and oral debates with Ed Masoro over the validity of *in vitro* cellular senescence as a model for the aging of intact organisms. Ed intends to discuss the science of these debates, so I'll restrict my own comments to those of a more personal nature. I'll always remember and cherish so many images associated with Vince and Ed heatedly going at one another or speaking privately with me about one another's differing views on this issue. Vince and Ed without exception challenged each other's views not only with the utmost passion and intellectual rigor, but also with profound mutual respect, admiration, and affection for one another, and always without a trace of bitterness. What an exquisite display of understanding and appreciation of the power of good science on both their parts!

Another of these intimate moments occurred in the context of Vince's conviction that good science includes responsibility to deal openly with the one or more weaknesses inherent to every experimental model. In particular I refer you to his research paper (2) that demonstrates the absence of any relationship between the replicative ability of cells in culture and the age of the donor. I vividly recall private conversations with Vince when he told me about colleagues who preferred that he not publish such data in fear of possible negative political consequences for federal funding of research on cellular senescence. However, Vince actually identified this publication as his favorite because it forced him to test his perception of scientific integrity. He realized immediately that

the implications of these studies are relatively minor to aging research but crucial to experimental design. What better testimony to Vince's character, as well as his understanding of and value for what it takes to do good science!

The final intimate moment that I shared with Vince was my attendance at his funeral mass in the local church where his family prays in suburban Philadelphia. I can count on the fingers of one hand the number of times during my adult life when I cried. My tears flowed freely that morning. However, they truly were not tears of grief, but instead represented the wonderful memories of our friendship. I listened as one of his daughters, Meg, delivered the eulogy. I also overheard several of the neighbors and other non-scientific acquaintances discussing their own memories of him. In every case the person described was the same Vince Cristofalo that I knew. Every one of them was aware of Vince's dedication to the hard work it takes to pursue scientific truth, and everyone in their own way loved him for it. I truly miss Vince's physical presence. However, the pride and joy of the memory of our friendship, as well as the opportunity to contribute to this celebration of his many accomplishments, transcends any need to mourn his loss.

Edward Masoro

Vince and I argued for 35 years over the relevance of replicative senescence of cells in culture to organismic aging. Although at times our arguments were heated, we developed a deep and enduring friendship, and my respect grew ever greater for him as a scientist.

I met Vince in 1970 at the first meeting of what became known in some circles as the "Philadelphia Mafia." Dick Adelman learned that I had just received funding from the NIH for an aging project, and since he and Vince were also starting NIH-funded aging research, he felt we should all get together periodically. That first meeting was held at the Wistar Institute. Because of his experiences in the Hayflick laboratory, Vince was knowledgeable about aging and Dick had formal gerontologic training. However, I knew almost nothing about aging. I had been working for many years on lipid metabolism when I was invited by the staff of the Aging Branch of the National Institute of Child Health and Human Development (NICHD) to attend a workshop on aging. At that meeting, the NICHD staff suggested that I apply for a grant to study lipid metabolism and aging - and I did. That the proposal was funded probably attests to the fact that the staff was desperate to get scientists interested in lipid metabolism into aging research.

At that first meeting, each of us presented a summary of our research project. I was puzzled by Vince's presentation, but reluctant to question him and thereby reveal my ignorance. By the second meeting, I was comfortable enough to ask Vince questions about his research; he recognized my lack of knowledge and suggested that I read a recent paper by Hayflick (3). The following statement in that paper served as the hypothesis underlying Vince's research: "Consequently, we have proposed that the finite lifetime of diploid cell strains *in vitro* may be the cellular expression of senescence so well known at the level of the whole animal." I told Vince that I thought such a hypothesis was farfetched and should not be a basis for the elegant biochemical analyses he planned to use to explore this *in vitro* model. Although we disagreed, our discussions were not heated.

In the autumn of 1973, I left Philadelphia for the University of Texas Health Science Center at San Antonio. Of course, this geographic separation greatly decreased the level of our interactions. However, we occasionally talked on the telephone about a variety of matters, including some further discussion of the relevance of the cellular senescence model to organismic aging. Indeed, Vince suggested that I read a recent paper by Hayflick (4). That paper provided an expanded view, fleshing out the hypothesis: "It follows, therefore, that an understanding of the mechanism by which cultured normal cells lose their capacity to replicate could provide insights into the causes of decrements in other functional properties that are characteristic of nondividing cells and that may be even more direct causes of biological aging." I told Vince that I was impressed by the quality of the writing in that paper, but was no more convinced of the validity of this view than before I read the paper.

I think our interactions would have gradually faded if it weren't for Ettore Bergamini of the University of Pisa. He invited us to teach at his university as well as to participate in several scientific meetings he organized. They were held in a former seminary in Volterra, a mountain town in Tuscany. It was during our times together in Tuscany that we had extended and sometimes heated discussions. During one of those discussions, Vince invited me to spend a week in his laboratory at the Medical College of Pennsylvania. During that week, Vince had one of his technicians show me the laboratory procedures used in his cell senescence studies, and the rest of the time was spent in discussions such as those in Tuscany. Vince said he looked forward to our discussions because I presented issues that he had not encountered in Hayflick's or his own laboratory. I emphasized that his elegant research was aimed solely at determining the molecular basis of the limited replicative potential of cells in culture, rather than testing the hypothesis that this model served as an *in vitro* model of organismic aging. This led him to suggest that we do a cell senescence experiment comparing cells from the skin of *ad libitum*-fed and caloric-restricted rats. I told him that I had contacted Jim Smith regarding such an experiment and sent Jim skin biopsies but that nothing had come of it. Vince said he felt he could do it and gave me detailed instructions on how to do the biopsies, pack them and send them to him.

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The major finding of this study was that cultured skin fibroblasts from the rats on a caloric-restriction regimen exhibit the same replicative potential as those from *ad libitum*-animals (5). I was elated by these findings until Vince pointed out that the results could be as likely due to caloric restriction not retarding aging as to the cellular senescence in culture not being a valid model of organismic senescence. This possibility led me to more thoroughly evaluate the caloric restriction model, particularly in recent years, and indeed, further evidence is needed to clearly establish that caloric restriction slows the aging processes.

At about this time, I was asked by the American Physiological Society to edit a volume on aging in their Handbook of Physiology series, a volume long overdue since information on aging had been conspicuously lacking in that prestigious series of handbooks. I invited Vince to write the chapter on the cell culture senescence model. He and his co-author, Robert Pignolo, wrote an excellent chapter (6). Not only did they discuss the cell senescence model, but also provided a brilliant discussion of the use of models in general in biology. I strongly recommend this chapter to those who have not yet read it.

Vince carefully reconsidered the published evidence in support of cell senescence in culture as a model of organismic aging, and he concluded there had been a lack of rigor in the studies indicating an inverse relationship between the age of the human donors and the replicative capacity of their cells in culture. He and his colleagues then carried out a study aimed at eliminating the deficiencies in the earlier work and, in a paper published in 1998, reported no relationship between the age of healthy donors and the replicative capacity of their cultured fibroblasts (2). As Dick Adelman just said, Vince was very excited by this finding and in 2001, when asked to identify which of his many papers he most valued, he chose that 1998 paper (7).

Vince was also concerned about the quality of the study of Rohme (8), which showed a positive correlation between the life span of a species and the replicative potential of its fibroblasts in culture. In a carefully designed study, Vince and his colleagues reinvestigated this issue and reported in 2005 that cellular replication in culture correlates with species body size and not with longevity (9). He was also excited by this finding and told me about it about a year before it was published. Vince truly believed in testing hypotheses with the intent of falsifying them rather than seeking support for them as his primary aim.

Our last discussion relative to the cell culture model occurred at the 2005 annual meeting of the Gerontological Society of America. For us, it was a remarkable interaction because it was primarily a resolution of our differences rather than further disagreement. Vince made the point that early passage cells from subjects of varying ages provide a valuable model for the study of aging. I responded that, of course, I recognized that *in vitro* models often provide valuable leads, but ultimately such leads have to be verified by *in vivo* studies. As a case in point, I mentioned Brian Merry's concern (10) that it is widely held that caloric restriction decreases the generation of reactive oxygen species, even though such a view is based solely on studies with isolated mitochondria. Vince agreed that there is a tendency to believe that what is found *in vitro* also occurs *in vivo*, and that this is a problem that needs to be addressed.

In regard to research on cell culture replicative senescence, Vince said he felt that such studies have and will provide invaluable gerontological information because a loss of cell number, hyperplasia and neoplasia are major problems for the aging organism. I agreed.

Leonard Hayflick

I will not reiterate the important scientific accomplishments that have been made by Vince because my colleagues, who have preceded me, have already described those contributions eloquently. Rather, I will mention a few anecdotes that will speak more directly to the personality of this man.

I first met Vince Cristofalo when he was brought to my laboratory at the Wistar Institute in 1963 by my colleague, Dr. David Kritchevsky. Unknown to me, Vince was a candidate for a position at the institute.

I will quote Vince who described in print that first encounter as follows (11):

"I knew about Leonard Hayflick and his aging cells both from the scientific literature and from various reports in the lay Philadelphia press. But, there I was looking upon this man, sitting at the desk in the center of his laboratory, with people bustling to and fro on all sides of him, going to the incubators or to the sterile rooms. Seemingly unperturbed by this frantic activity, Leonard was dictating letters on a tape recorder. My host, David Kritchevsky, interrupted him to introduce me. Len looked, for all the world, annoyed at the interruption. His demeanor signaled that he wished I would go away and not return. Nevertheless, he was minimally cordial, he gave me a reprint of his 1961 paper with Paul Moorhead and returned to his dictation."

"Despite this less than compelling introduction, I joined the Wistar Institute staff a month or so later. Since my assignment was to work on the limited replicative life span of human cells in culture, I spent many hours in Len's lab. (Parenthetically I should say that I was never told by anyone that Vince had been given this assignment). His technicians taught me the technology of cell culture. Conversations with Len, although sometimes painfully abrupt, gave me insights into his thinking and an introduction to the biology of aging."

"The first impressions that I had of Len's aloof demeanor soon changed to appreciation of his thoughtful,

penetrating style, and his focus on his work and its importance. I learned a great deal from him and about him in those days that we were together at Wistar, and we have remained friends and colleagues over these nearly forty years."

I do not find fault with anything that Vince has described about our initial meeting. However, what Vince did not know when he first appeared in my lab was that he did not enter any ordinary research laboratory. He entered what is more accurately described either as a boiler room or the cab of a runaway steam engine.

My paper on the finite lifetime of normal human cells had just been published and because we described that these cells had the broadest human virus spectrum of any cell population then known, the phone calls and mail brought hundreds of requests for starter cultures and how to grow them.

We also suggested that because these cells had no indigenous viruses present, and that they could be characterized before use, unlike the dangerous primary monkey kidney cells then used by Salk and Sabin for poliomyelitis vaccine production, virtually every polio vaccine manufacturer in the world was asking for starter cultures. In addition, many manufacturers had sent people to my lab to learn the technique that we described for producing polio vaccines in normal human cells.

As if that were not enough, people working in human virus diagnostic labs world wide also sought starter cultures in order to detect human viruses in the populations they served. Add to this the interest in obtaining starter cultures and learning how to grow them by those working in the field of aging and you will get some ideal of the chaos that greeted Vince when he first entered my lab.

But that was not all, in the same year I had discovered that the human disease called Primary Atypical Pneumonia or "walking pneumonia" was not caused by a virus, as had been previously thought, but that it was caused by an organism called a mycoplasma – the smallest free living microorganism. I named this organism Mycoplasma pneumoniae and, because it was attenuated, dozens more vaccine manufacturers and researchers were also demanding cultures of this organism.

After a few days in my lab when Vince realized why I had been so short with him, our friendship blossomed and he took his place among the many other trainees in my lab.

Vince then became the first person to mount a program in order to understand the biochemistry of cultured normal human cells. In those days it was called biochemistry until it yielded later to the more glamorous term "molecular biology." The importance of glamorous and prestigious labels is alive and well in science.

Vince did a magnificent job in describing the fundamental chemistry of the normal human cells which included their metabolism, nutritional requirements and, in later years, their properties related to aging.

Over the years my friendship with Vince increased further as we both found ourselves together at many professional meetings, committees, and courses.

In time I discovered that Vince had joined a group called the Philadelphia Mafia that included Jay Roberts, Ed Masoro, George Baker, and Dick Adelman.

I could never understand why Vince would join an organization in which he would be characterized as a Mafioso. His quiet, dignified and thoughtful demeanor never reminded me of Don Corlione or Tony Soprano. I hasten to add that none of the other members, who, curiously, took pride in calling themselves Mafioso, appeared to me to share those characteristics either. None of them came close to resembling my West Philadelphia neighborhood Don or my regional Capo.

Although this group of Philadelphia Mafioso took pride in labeling themselves as such, they have never revealed who among them is the Capo di Tutti i Capi.

It is this mystery, and the possibility that maybe there was something in the character of all of the members that was dark and that had been successfully concealed from me, that I decided to not challenge the group by asking them why I was never offered membership.

I did not then, and still do not, relish being kissed on both cheeks by any of them nor do I want to find a severed, bloody horse's head in my bed.

When I moved to California and our contacts were reduced I had made it a practice to arrange lunch with Vince often when I came to Philadelphia to see my now 101 year old mother. In the last few years we met two or three times a year at, fittingly, an Italian restaurant across the street from my mother's apartment building. Over pasta and Chianti we both gossiped about mutual friends – some of them are here today – or we had intense discussions about various aspects of biogerontological research, politics and funding.

Although I knew that Vince had had an episode or two of minor health problems I did not know that in the beginning of this year he had encountered a more serious health problem. Peggy, his wife, told me a few weeks ago that it was his decision not to reveal this problem to anyone outside of his immediate family. I suppose that Vince did not want to worry his friends nor did he want to draw attention to himself. These qualities were typical of Vince.

I will miss our Philadelphia lunches and the man whose thoughtful demeanor, high personal standards and brilliant scientific insights have benefited me and all who have had the good fortune of including him in their lives.

Christian Sell

A consideration of Vincent Cristofalo's life must include an analysis of some of his scientific work along with a discussion of its implications for aging research in general.

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Vince's influence on the field of aging is as broad as his career was long although arguably the most influential portions of his work may be those relating to the correlations between replicative lifespan and the age of the donor or species lifespan. These 2 attractive concepts, that cellular lifespan decreases with donor age and that species lifespan is reflected by cellular lifespan *in vitro* were widely quoted in the field, although little experimental evidence existed to support them. The concept that replicative lifespan decreased as a function of donor age (12, 13) had been called into question on at least one occasion (14) and Vince firmly believed that it was the responsibility of a scientist to clarify ambiguities related to their work. It was this sense of responsibility that compelled Vince to critically reexamine the experimental evidence to both of these concepts although this was not a popular cause. Vince and his group (at this time headed by RG Allen and Bob Pignolo) began to test the replicative lifespan of a series of human fibroblast lines stored at the Coriell Institute in Camden NJ in collaboration with Dr. Beck at the Coriell. The cell cultures used were derived from the Baltimore Longitudinal Study of Aging and only healthy donors were included. Following a rigorous series of tests to determine intra and inter assay variability, the group determined that there was no correlation between donor age and replicative lifespan of normal human fibroblasts in culture (2).

A reassessment of the second concept, that species lifespan is reflected by the lifespan of cells in culture was more difficult to test due to the paucity of primary cultures from a variety of species with differing lifespans. There were 2 reports in the literature, one supporting a relationship between species lifespan and replicative potential and one indicating no relationship (8, 15). Simone Lorenzini, who was working with Vince at this time, collected a series of skin biopsies from a collection of 11 mammalian species and established 59 cultures. The cultures were carefully standardized in terms of culture conditions, media composition, serum lot, etc. The values for species age and mean adult body mass were only taken from verified sources in an attempt to provide the most accurate information. The lifespan of the cultures were established through an analysis of growth rate, cell size, and karyotype. The statistical analysis of the results required consideration of the influence of body mass due to the positive relationship between body mass and lifespan across species. Bivariate analysis and logarithmic data transformation revealed that the relationship between replicative capacity and lifespan was relatively weak and that following correction for body size there was no significant correlation. However, the relationship between replicative lifespan and body size remained significant following correction for lifespan. Thus, replicative lifespan correlated with body size and not species lifespan (9).

Attention then turned to the possibility that other functional characteristics of cells derived from different species may reflect differences in lifespan. Vince and Dr. Lorenzini began to examine DNA damage recognition in collaboration with Dr. Thomas Stamato of the Lankenau Institute of Medical Research. They found that there was a significant difference in the ability of cells to recognize double strand breaks in DNA that correlated strongly with lifespan but not body size. In fact, the ability to form complexes at the site of a double strand break increased exponentially with increasing lifespan. These intriguing results strongly suggest that fundamental structural differences exist in cells from different species. It would be exactly this type of difference that one might predict to be related to lifespan. Consider that the vulnerability to disease increases at an exponential rate in most species which would suggest that mechanisms that prevent the increased vulnerability might also require an exponential increase in efficiency to increase lifespan.

In addition to his scientific contributions, Vince was involved in efforts to establish programs related to aging throughout his career. At the University of Pennsylvania he established the Institute on Aging that continues to support aging related research. At the Medical College of Pennsylvania, Vince established the Center for Gerontological Research which broadly supported aging related research and education. This effort was curtailed by the eventual bankruptcy of the Allegheny Health System which engulfed the Medical College of Pennsylvania. Vince then moved his efforts to the Lankenau Institute for Medical Research where he became the President and CEO. Vince moved the institution towards aging related research until his eventual retirement. These efforts were in addition to Vince's work on behalf of the Gerontological Society of America and the American Federation for Aging Research, among others. Vince's efforts as a researcher, educator and organizer of aging related research will certainly be remembered and the impact of his life on the scientific community in general provides a testament that transcends any that we could provide here.

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