Frank Chytil (1924–2010)

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On January 31, 2010, the Czech “mafia” lost one of its most colorful soldiers. Frank Chytil was extremely proud of his Czech heritage and fiercely loyal to the many other Czech scientists who made their way to the West, whom he lovingly referred to as the “Czech mafia.” Having grown up under a communist regime, Frank had to endure obstacles that most us cannot imagine, yet he viewed many of those obstacles as “lucky” circumstances that led to his subsequent productive research career in the US.

Childhood and education in Prague
Frank was born in Prague, Czechoslovakia, on August 28, 1924, and his first 14 years would be considered a typical middle-class childhood. The youngest of 3 children, Frank attended grade school and the public gymnasium (equivalent to high school) in Prague. It was during his time at the gymnasium that Frank was introduced to one of his lifelong loves: basketball. Frank played for the premier team in Prague, AC Sparta, and was good enough to be named to the 1948 Czechoslovak Olympic team. Things began to change for Frank and his family in 1939, when the Nazis occupied the western part of Czechoslovakia, including Prague. Because Frank’s grandmother was Jewish, his father lost his banking job, and several relatives were sent to labor camps during the war. Frank was allowed to graduate from the gymnasium in 1943, after which he was assigned to work in the chemical laboratory of an airplane factory. In between his assays for nickel in steel, Frank found an unused polarograph in the laboratory that led him, unexpectedly, to his second lifelong love: biochemistry. To learn how to use the instrument, Frank sought out J. Heyrovsky, the inventor of polarography, who was then a professor at Charles University in Prague. Among several useful pieces of advice, Heyrovsky recommended that Frank should study biochemistry. This phase of Frank’s education ended when the U.S. Air Force bombed the factory and his polarograph, an event he took great satisfaction from!

1945 brought the end of the war, the liberation of Czechoslovakia, and the reopening of the universities. Biochemistry was not offered, so Frank earned a degree in analytical metallurgy from the School of Chemical Technology in Prague and landed a job as an analytical chemist with the endocrinologist Josef Charvat. Charvat was studying stress, and he taught Frank about glucocorticoids and the value of whole animal studies. Frank used microbiological assays to measure the stress response of sulfur-containing amino acids in blood, and this work triggered his interest in steroid hormones. This was also about the time Frank discovered his third lifelong love, Lucie Scheinostova, whom he married in 1949. Unfortunately, the Communist putsch of 1948 had transformed Czechoslovakia into a dictatorship. The Czech Communists jailed Lucie’s father, removed Lucie from her post at the university and sent her to work in a bakery, confiscated Frank’s parents’ house, and evicted Frank and Lucie from their home. Frank was scheduled to be drafted into the Communist army, destined to serve in the coal mines. Charvat helped Frank defer his military service by arranging for him to enter doctoral studies under Arnost Kleinzeller in the Department of Fermentation Chemistry in the School of Chemical Technology. Frank’s work with Charvat and Kleinzeller was the basis of his Doctorate of Rerum Technicarum in 1952.

Early career in Communist Czechoslovakia
Following his doctorate, Frank continued in Kleinzeller’s laboratory, developing a fermentation process for vitamin B-12 production. Once again, the Communists had other ideas. Kleinzeller (a Jew) was suddenly dismissed from his position and Frank lost his job. And once again, as Frank would say, “I was lucky.” He landed a position with Otakar Poupa in the Institute of Nutrition, where he studied the metabolic consequences of the adaptation of rats to trauma. These experiments required that Frank capture wild rats from the Prague sewer system and carry them in cages on public transportation to the laboratory. It is safe to say that the Institute of Nutrition did not have the kind of animal care committees we have today!
Frank’s work was quite productive and he was soon given the independence of his own project supported by the Institute. His previous experience with Charvat led him to pursue Gene Knox’s observation that tryptophan pyrrolyase (dioxygenase) is induced by adrenal cortical hormones (1). Frank showed that switching rats to a high-protein diet also induced the activity of this enzyme (2). This work caught the attention of Nate Kaplan at Brandeis University, and Frank eventually obtained permission from the local Communist Party to join Kaplan’s laboratory in Boston, but only for 6 months, and without his family; his wife and their 3 children “had to remain in Prague as hostages.” (3). Upon his return to Prague, Frank was subjected to several months of interrogation by the secret police, and this experience convinced him and Lucie that it was time to escape from the “paradise of communism.” They planned their escape with Jan Lukas, the famed Czech photographer, and his family. Because they were not allowed to travel as a family, they had to split up. Frank had obtained permission to take his 11-year-old son, Frank Jr., with him to the 1965 Biochemical Congress of European Biochemists in Vienna. Lucie gained permission to go for a short holiday to Yugoslavia with their 2 young daughters, Anna and Helena (9 and 7 y old). They departed on the same day, and a week later, Lucie and the girls, with the help of Jan and Milena Lukas, successfully crossed the border between Yugoslavia and Italy. Lucie and the girls spent 3 months in a refugee/detention camp in Italy before the family was finally reunited.

**Life in the US**

Kaplan provided Frank a temporary position in the Department of Biochemistry at Brandeis, and Frank was soon offered an independent position at the Southwest Foundation for Research and Education in San Antonio, TX. There he continued his studies on the role of cAMP in the activation of tryptophan dioxygenase (4). In 1969, Frank was offered a junior faculty position in the Department of Medicine at Vanderbilt University School of Medicine in Nashville, TN. Bert O’Malley had just moved to Vanderbilt and was assembling a group to study the mechanisms of steroid hormone action. Frank joined a talented group that included Tony Means, David Toft, Thomas Spelsberg, Chuck Strott, Bob Northcutt, Jeff Rosen, and Bill Schroeder. Frank’s early work at Vanderbilt involved immunochromatographic analyses of chromatin and the tissue specificity of non-histone nuclear proteins (5). This technique was then also used to demonstrate changes in chromatin composition in the oviduct of chicks administered progesterone (6). These studies complemented the exciting work going on in all the laboratories of O’Malley’s Vanderbilt team, eventually leading to the discovery of steroid receptors (7,8).

**Vitamin A**

In 1973, Bert O’Malley moved to Texas to start a new Department of Cell Biology at Baylor Medical School. He took many of the members of the steroid group with him and invited Frank to move to Houston. However, Frank decided that he had moved his family enough and chose to stay at Vanderbilt. This turned out to be another “lucky” decision, for Vanderbilt, for Frank, and for the field of vitamin A research.

Frank’s longstanding interest in regulation of gene expression had led him to consider that certain nutrients might also be involved in such regulation. Based in part on his prior observation of vitamin A-deficient farm animals in Czechoslovakia and recent published accounts of alterations of RNA metabolism in laboratory animals made deficient in vitamin A, Frank selected this vitamin as a prime candidate to be examined for regulation of gene expression. He had received a joint appointment in the Department of Biochemistry and attracted his first graduate student, Mark Bashor. They planned to examine changes in chromatin composition caused by vitamin A deficiency as he had done previously for changes upon progesterone action. The prevailing theory at that time was that retinol worked as a coenzyme for enzymes involved in protein glycosylation, but, for Frank, this seemed insufficient to explain the dramatic changes caused by deficiency. Frank’s experience with the O’Malley group led him and Mark to postulate that vitamin A worked via a mechanism similar to that being elucidated for steroid hormones.

A problem arose quickly in their first experiments: the purchased rats steadfastly refused to show signs of deficiency on the vitamin A-deficient diet. Weeks were passing with nothing for Mark to do. To fill in the time while waiting for deficiency to show itself, they recruited Dave Toft to help them look for a binding component for retinol. Toft and Jack Gorski had developed a technique using sucrose-gradient ultracentrifugation separation to identify the estrogen receptor protein. By using radiolabeled retinol with this technique, they demonstrated a soluble protein, present in a number of rat tissues, that bound retinol with high affinity and specificity. While this was not the postulated nuclear receptor protein (nor was it ever purported to be by Frank and his colleagues), the publication describing this discovery (9) clearly laid out the driving hypothesis that the mechanism of action of vitamin A-active compounds would be the same as that being described for steroid hormones. Briefly, they postulated that a vitamin A-active molecule would bind to a protein (receptor) and this complex would bind to DNA in a way that activated or repressed transcription of specific genes. This groundbreaking hypothesis for vitamin A action has been thoroughly tested and is now well established as the primary mechanism by which vitamin A regulates gene expression. Indeed, a PubMed search on “retinoic acid” and “receptor” gives the 1973 Bashor et al. (9) paper as the first to be retrieved, followed by 11,000 more! The actual nuclear receptor protein(s) remained elusive until they were described by work from the laboratories of Ron Evans and Pierre Chambon, both publishing in 1987 (10,11), 14 years after Frank’s prediction.

The binding activity described in that first publication proved to be only the first intracellular binding protein for vitamin A-active compounds, cellular retinol-binding protein (CRBP). David Ong, who received his PhD in 1970, was fortunate to join Frank’s laboratory in late 1973. Together they described the second member of the cellular vitamin A-binding proteins, cellular retinoic-acid binding protein, or CRABP, discovered during the purification of CRBP (12). Both are members of the fatty acid-binding protein super-family. Other members followed. This early work had opened a second area of research, still very much active today, on the movement and metabolism of retinoid compounds within the cell as directed by such proteins.

These achievements were recognized when the AIN granted their highest award for basic research, the Osborne and Mendel Award, to Frank and his colleague David Ong in 1983. This was the first time, 34 years from its initiation, that the award was given to a collaborative team rather than an individual. It is indicative of Frank’s lifelong respect for and encouragement of younger scientists. Other teams were recognized later by this same award, making Frank a pioneer here as well! In 1984, Frank was named the General Foods Distinguished Professor in Nutrition at Vanderbilt.
Vitamin A and Premature Infants

Frank’s wide-ranging interests and knowledge of previous observations came to the forefront as that initial discovery was quickly followed by studies on the binding proteins that predicted the important roles of these proteins and vitamin A in cancer (13) and in development, particularly that of the lung (14). This interest in the role of vitamin A in lung development soon led to a further area of notable achievement for Frank.

Frank was always interested in how his basic research might be translated into clinical practice. An area of expertise at Vanderbilt was the care of very low birth weight neonates. Mildred Stahlman, MD, had established the world’s first newborn intensive care unit, pioneered the use of respiratory therapy in these tiny infants, and assembled an excellent team dedicated to improving their care (see http://www.nlm.nih.gov/locallegends/Biographies/Stahlman_Mildred.html). These infants were frequently born with, or soon encountered, serious lung problems because of incomplete development and/or damage from the high oxygen levels required for successful respiratory therapy. Frank recognized that vitamin A might be a factor to be considered for these infants and began a collaboration with Stahlman’s team.

This proved to be a very productive collaboration. Frank continued basic research studies on lung development in rats and, with Stahlman and her team, initiated a series of investigations concerning human infants. These carefully conducted studies built a compelling story of frequent vitamin A deficiency in premature infants (15,16), failure of the then-current method of supplementation to raise their vitamin A status (17), and marked improvement of lung function when successful supplementation of vitamin A was accomplished by i.m. injection (18).

Frank was named a Fellow of the American Society for Nutrition in 1991, and he received the Lederle award in Human Nutrition (now the Centrum Center for Nutrition Science award) in 1993 for his pioneering work in this area. Of all of his research accomplishments, Frank was most proud of this work.

Lasting legacy

As pioneering as these discoveries were, perhaps Frank’s greatest legacy is the impact he had on the people who passed through his laboratory. Frank never had a particularly large group, usually just 2 or 3 graduate students, an occasional post doc, a technician or two, and of course his wife Lucie, who worked side by side with him for 22 years. The following is a partial list of people who trained in Frank’s laboratory: George Catignani, Mark Bashor, David Ong, Louise Canfield, Sachiko Takase, T.C. Tsai, Elizabeth Wilson, Brian McGuire, Dean Appling, Gene Liu, Masahide Omori, Steve Porter, David Sherman, D. G. Stump, and R. U. Haq. Many of these people have gone on to make important contributions of their own in nutrition and biochemistry. Frank encouraged free thinking and risk taking in our research but demanded precision and reproducibility. Frank instilled in all of us the importance of integrity and history in the scientific enterprise. Frank Chytil will be missed; indeed, those of us who were privileged to know and work with him are truly the lucky ones.

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Literature Cited