Retrospective:
Helmut Beinert (1913-2007): A Nonagenerian Par Excellence!

The man whom many would identify as the founder and first proponent of the use of electron paramagnetic resonance (EPR) spectroscopy in biological systems, Helmut Beinert, passed away at the age 94, after a brief illness. His excellent health permitted him to visit his office almost every day at the (former) Institute for Enzyme Research of the University of Wisconsin, where he spent many years of his professional life. His state-of-the-art science had earned him an invitation as an invited speaker for the upcoming 2008 Gordon Research Conference on Iron-Sulfur proteins. As a true pioneer in Bioinorganic Chemistry, and the most prominent researcher in the area of Fe-S proteins for many years, he had been asked to speak on the history of these important proteins.

Beinert helped to install the first EPR spectrometer, equipped with liquid nitrogen cooling to perform experiments down to 77 Kelvin, at the University of Konstanz in the late 1960s. Although the University of Konstanz competed for the recruitment of Beinert, the University of Wisconsin at Madison was able to retain him there where he performed his research for a major portion of his professional life. The Madison campus became the destination for anyone interested in studying metal-containing enzymes, recording EPR spectra of biological samples below 10 K, capturing catalytic intermediates within milliseconds using rapid-freeze techniques, or squeezing the most information from milligram quantities of meticulously prepared proteins in the absence of dioxygen. His laboratory had the appearance of an engineering shop where he and his erstwhile colleague, Ray Hansen, were able to design the instrumentation and develop the techniques required to address biological problems.

Beinert's research career was noted not only by the significance of his discoveries, but also by the fact that he was one of the few well known scientists who consistently performed research with his own hands. In the 1980s, during a period when retirement was mandatory, with the assistance of the administration, Bettie Sue Masters, as Chairman of Biochemistry, recruited Beinert to the Medical College of Wisconsin as a Distinguished Scholar-in-Residence, providing him with salary and endowment support for his NIH-supported laboratory activities. He brought with him Mary Claire Kennedy, S.S.J., who, side-by-side with Beinert, conducted their premier studies on aconitase[1].

In a 1992 paper, they and their collaborators revealed a second aconitase, found only in the cytosol of mammalian tissues, which in its apo-form functions as an iron regulatory protein (IRP1). They characterized the beef liver cytosolic aconitase and demonstrated it to be active in its [4Fe-4S] form with a turnover number similar to that of the mitochondrial aconitase. However, the EPR spectra of the two enzymes were shown to be markedly different, whereas their amino acid composition, molecular weight, isoelectric point, and the sequences of six random peptides clearly showed their physicochemical and structural characteristics to be identical to those of IRP1, but that cytosolic aconitase is distinctly different from mitochondrial aconitase. These experiments revealed a new role for aconitase and a mechanism by which it could be involved in intracellular Fe homeostasis. Mössbauer experiments on Fe-S centers of a number of different proteins, including aconitase, in collaboration with Eckard Münck, revealed mechanistic aspects of the role of the Fe-S clusters in these proteins[2].

Because Beinert collaborated with physicists and explored their advanced techniques for the analysis of complex biomolecules, he was able to address many difficult biological problems. Utilizing EPR, he was able to

address the copper sites in cytochrome oxidase in collaboration with Richard Sands[3]. With both Sands and Münck, physicists by training, Beinert performed interdisciplinary research at the highest level. He also studied the fatty acyl-CoA dehydrogenases in pig liver mitochondria, founding yet another field in intermediary metabolism, which plays a major role in human health[4].

The original acyl-CoA dehydrogenase, medium chain acyl-CoA dehydrogenase (it was then called general acyl-CoA dehydrogenase), was discovered by David Green's group and, as Beinert wrote[4], Green "farmed out" the acyl-CoA dehydrogenase project to him for purification and characterization, which resulted in a series of papers that describe all of the fundamental enzymological aspects of acyl-CoA dehydrogenases. He said "Unexpectedly, but not unfortunately, the acyl-CoA dehydrogenases were a starting point for me into quite a different direction"[4]. During the course of these studies, he discovered long chain fatty acid-specific acyl-CoA dehydrogenase, and later electron-transferring flavoprotein (ETF) and ETF-ubiquinone oxidoreductase that link fatty acid metabolism to the main mitochondrial respiratory chain. Those of us who study enzymes involved in βoxidation marveled at Beinert's biochemical instinct and insights: all of the biochemical properties of the acyl-CoA dehydrogenases and ETF we now know were anticipated by Beinert in the 1950s!

Beinert was born in Lahr, a small town in Baden, Germany, on November 17, 1913. In 1955, he became a U.S. citizen. He received his Abitur in 1932 at a classical German Gymnasium in Heidelberg, Germany, graduating in Greek and Latin. "I was certainly not predestined or even prepared to enter the world of frontline biochemical research," recounted Beinert. "In the close neighborhood, there was the Kaiser-Wilhelm-Institute (KWI), and one day the children of two KWI directors, Prof. Meyerhof (Physiology) and Prof. Hausser (Physics), suddenly appeared in our school"[5]. During his final exams, a rather unique meeting at the KWI occurred entitled, "Lectures and Demonstrations about Foundations and Problems of Biological Oxidation Processes." With almost all the great names in the field in attendance: Warburg, Keilin, Haldane, Krebs, Kuhn, and Meyerhof, it was most likely a momentous event. Beinert then began studying chemistry in Heidelberg and Leipzig and, in 1943, obtained his doctoral degree from the University of Leipzig, while performing his thesis research in the laboratory of Richard Kuhn, at the KWI for Medical Research in Heidelberg.

After working there as a Research Associate until 1945[5], Beinert left for the U.S. He spent several years with the U.S. Air Force School of Aviation Medicine in Randolph, Texas. He then joined the Institute for Enzyme Research at the University of Wisconsin in Madison in 1950, where he became a full professor in 1962. He stayed in Madison until his retirement in 1985 at which time he was recruited to the Medical College of Wisconsin as a Distinguished Scholar-in-Residence. One of the attractions there was the National Biomedical ESR Center, under the direction of James S. Hyde. Beinert served on the EPR Center Steering Committee as a member until his death.

Beinert received many honors and awards during his career, including induction into the National Academy of Sciences in 1980 and the Keilin Medal from the British Biochemical Society in 1985, followed by the Warburg Medal from the German Society for Biological Chemistry in 1994. In the same year, Beinert received the first Honorary Doctoral Degree from the Faculty of Biology from the University of Konstanz.

Throughout his prolific career, Beinert contributed many discoveries and insights to the field of metalloenzymes, redox enzymology, bioenergetics, and Fe homeostasis. His research has formed the basis of much of our information in the field of biological oxidations in the modern textbooks of biochemistry. Beinert is survived by a daughter, Isabel, and son, Hannes. His wife, Elisabeth, passed away in April, 2005.

Beinert, who relished and, in fact, insisted upon remaining involved in his own experiments, will be sorely missed by his many admirers and colleagues. Vivid in our memories is the virtually photographic image of Beinert and Kennedy, side-by-side, performing anaerobic titrations with their custom-constructed equipment as though in another world and another time. We all knew that what would result was quite futuristic![a]

Respectfully,
Bettie Sue Masters, Ph.D.
Mary Claire Kennedy, S.S.J., Ph.D.
Jung-Ja P. Kim, Ph.D.
Peter H. Kroneck, Ph.D.

REFERENCES:

1. Beinert, H., and Kennedy, M. C. (1989) *Eur. J. Biochem.* **186,** 5.
2. Beinert, H., Holm, R. H., and Münck, E. (1997) *Science* **277,** 653.
3. Beinert, H., Griffiths, D. E., Wharton, D. C., and Sands, R. H. (1962) *J. Biol. Chem.* **237,** 2337.
4. Beinert, H. (1988) in *Fatty Acid Oxidation: Clinical, Biochemical, and Molecular Aspects* (Tanaka, K., and Coates, P., eds) p. 1-22, Alan R. Liss, New York.
5. Beinert, H. (1999) in *Flavins and Flavoproteins* (Ghisla, S., Kroneck, P., Macheroux, P., and Sund, H., eds) p. 3, R. Weber Agency for Scientific Publications, Berlin.

FOOTNOTE:

a. For more information on Beinert's research, see his *JBC* Reflection (Beinert, H. (2002) *J. Biol. Chem.* **277**, 37967-37972)