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Jeremy R. Knowles (1935–2008)

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Jeremy R. Knowles. Image courtesy of Jon Chase/Harvard News Office.

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Jeremy Randall Knowles was born on 28 April 1935 in the market town of Rugby, England. Rugby is a one-hour drive from the campus of Oxford University, an environ that was to be his home for nearly 40 years. After two years of service as a Pilot Officer in the Royal Air Force, Knowles matriculated at Oxford, where his father, like his father before him, was a professor. After receiving his B.A., First Class, Knowles carried on for a D.Phil. in the laboratory of a young physical organic chemist, Richard Norman. His doctoral research was on electronic effects on the rates of aromatic substitution reactions. At age 25, he accepted an offer to be a Research Lecturer at Oxford, and he married Jane Sheldon Davis.

In 1961, Knowles spent an epiphanic six months on leave at Caltech. While working with organic photochemist George Hammond (*cf*: Hammond's postulate), his conversations with Bryan Jones, now Emeritus University Professor at Toronto, led to a profound transformation. Jones, who like Knowles had received a D.Phil. from Oxford, was doing postdoctoral work with Carl Niemann on the surprisingly broad substrate specificity of α-chymotrypsin. As a graduate student, Knowles had been fixated on understanding why some reactions went 10 times as fast as others. At Caltech, he encountered enzyme-catalyzed reactions that went 1 million times faster than unaided ones! The allure of these biological catalysts was overwhelming to the young physical organic chemist (and led 30 years later to his well-known aphorism: "enzyme catalysis: not different, just better"). Hence, Knowles became an enzymologist, honing his skills and perceptions first on the substrate specificities of α-chymotrypsin and pepsin.

Soon, Knowles turned his attention to the opposite end of the specificity spectrum. α-Chymotrypsin and pepsin are nonspecific proteases that function in extracellular environments and accept a broad array of substrates. Knowles decided to sink his teeth instead into a highly specific enzyme of primary metabolism, believing that such a catalyst had much to reveal about how to perform a single task exquisitely well.

The choice of the glycolytic enzyme triosephosphate isomerase (affectionately named "TIM") was astute. The TIM reaction involves the interconversion of a single substrate and a single product with an overall equilibrium constant close to unity. These two attributes enable detailed characterization of the reaction in both the forward and the reverse direction. Moreover, the mechanism proceeds *via* an enediol intermediate, providing additional benefits. The existence of this intermediate allows solvent protons (or deuterons or tritions) to enter (or leave) the reaction from the middle, rather than only from the substrate or product. Discerning the energetics of this intermediate and its flanking transition states put ideas on reaction energetics to a more rigorous test.

In the early 1970s, the Knowles group carried out a series of 16 heroic experiments on the TIM reaction, each employing hydrogen isotopes in a distinct but incisive manner. Highly significant in the interpretation of the resultant data was John Albery, a physical chemist at Oxford. These long-term collaborators had an idyllic complementarity.

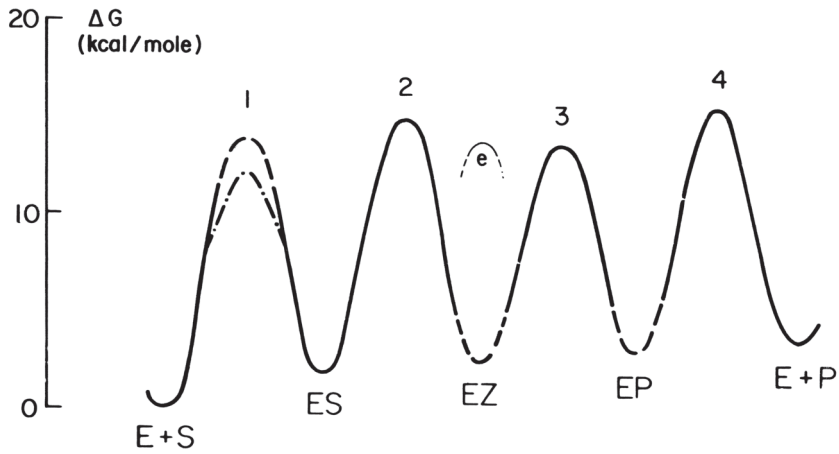

Figure 1. Free energy profile of the reaction catalyzed by triosephosphate isomerase (*1*). S is dihydroxyacetone phosphate, Z is the enediol intermediate, and P is D-glyceraldehyde 3-phosphate. Reprinted from (*1*). Copyright 1976 American Chemical Society.

Whereas Albery proffered a tome of dense algebra to substantiate the movement of a proton from one functional group to another, Knowles distilled the mathematical equations and their practical consequences down to a single squiggly line—a free energy profile. These profiles, which adorned the chalkboards of his office and laboratory, provided an elegant conceptual framework for intuiting the intricacies of catalysis.

In 1976, the results on TIM—algebra and squiggles—were reported in eight papers published consecutively in a single issue of the journal *Biochemistry*. The culmination was a landmark in the history of enzymology—the elucidation of the first free energy profile of an enzyme-catalyzed reaction (Figure 1) (*1*). A striking pattern emerged in this profile. The transition states were all of comparable free energy, as were the enzyme-bound intermediates. This equanimity led Albery and Knowles to penetrating insights about the evolution of biological catalysts. The mountains had eroded to a similar height, each having an incentive to lessen to the limit provided by physical diffusion but none having an incentive to be lesser still. The valleys had elevated to a similar depth to discourage the inordinate accumulation of any one intermediate (*cf*. Knowles' analogy to *Isaiah 40:4*). This elegant elucidation of "perfection" in enzymatic catalyses and its evolution is an immortal contribution to chemical biology (*2*).

Extended time at Illinois in 1962, Yale in 1969 and 1971, and Harvard in 1973 had instilled in Knowles a yearning to be in an American chemistry department, which were "more prepared to accept the fact that biological problems at the molecular level are fair meat for the chemist." He moved his research group to Harvard University in fall 1974, and, along with colleagues Konrad Bloch and Frank Westheimer, established the roots of chemical biology on that campus.

The transplanted Knowles team continued to make broad and seminal contributions. Important work on β-lactamases, their mechanism-based inhibitors (*e.g*., clavulanic acid and the carbapenems), and the means by which they entered the periplasmic space spanned the realms of organic chemistry and microbiology. Fascinating mechanisms were revealed for enzymes of the shikimic acid pathway, and intellectual seeds were planted for a "green chemistry" based on these plant/microbial enzymes. Much focus was on the stereochemistry of phosphoryl group transfer reactions, making use of chiral [^{16}O,^{17}O,^{18}O]phosphoryl groups and analyzing the stereochemical course of reactions by clever mass spectrometric and ^{31}P NMR spectroscopic methods. For example, the Knowles team used their chiral phosphate strategy to provide the first direct evidence (in 1982) for pseudorotation in the reaction of a phosphate monoester and (in 1988) of monomeric metaphosphate (that is, PO_3^-), which had long been sought as a solvated species.

Proline racemase provided an encore to TIM, along with two landmark discoveries. In a series of seven 1986 *Biochemistry* papers, the Knowles group and Albery revealed the consequences of "oversaturation", a regime in which the interconversion of distinct unliganded forms of the enzyme limits catalysis. They also described an elegant kinetic isotope experiment that reveals, without ambiguity, whether a reaction proceeds in a stepwise or concerted manner.

Though renowned as an enzymologist, Knowles made contributions to numerous other aspects of chemical biology. In 1968, he and Fred Richards (who was on a sabbatical from Yale) deduced the mechanism and products of glutaraldehyde-mediated cross-linking. In 1972, Knowles developed the method of photoaffinity labeling. The initial experiment used antibodies raised in rabbits against a 2-nitro-4-azidophenyl haptenic group. Analogous labeling experiments were performed with α-chymotrypsin, leading to the premonition that photoaffinity labeling was useful "both for the mapping of sites in homogeneous systems and for probing of the basis of heterogeneity in systems that are not." Work in 1980 used diazirines instead. Upon photolysis, diazirines yield carbenes, which are more reactive and less selective than are the nitrenes generated from arylazides.

At Harvard, the Knowles research team of 16 or so graduate students and postdoctorates brought chemical expertise to bear on these and other outstanding issues in biological chemistry. Always, he guided their efforts with both the question, "Are we illuminating existing problems, or merely creating a large number of new ones?" and the conviction that "one need not tell the world one has found another weed even though one was looking for a new hybrid rose." Accordingly, the density of gems in the Knowles bibliography is high. The inheritance of these values was evident at "Whither Enzymology?", a wonderful symposium in his honor on the occasion of his 60th birthday. There, former graduate students, postdoctorates, and collaborators celebrated his life, with John Albery providing a memorable postprandial retrospective. The December 1995 issue of *Bioorganic Chemistry* was dedicated to this occasion and includes a scholarly and comprehensive review of Knowles, scientist (*3*).

The Knowles wit likewise permeated his coworkers. Installments in the "Complaints and Suggestions Book" provided a humorous diary of life in his laboratory. CASB volumes transcended the Atlantic and served (for example) to teach the Americans that "pants" were worn underneath "trousers". A favorite entry was "John G's Hyperspatial Mutase Preparation Game", which described the saga of purifying wheat-germ phosphoglycerate mutase from its natural source. Square 3 of the game board was labeled, "You extract toasted wheat germ by error. Go back to start." The final square was labeled "∞".

The Knowles legacy extended beyond those fortunate to have trained in his laboratory. His charm could befriend in an instance, as well as disarm the most virulent of journal referees. His ability to entrance an audience was remarkable. His tales of the evisceration of an enzymatic reaction or bioorganic process with the precise tools of physical organic chemistry were crafted meticulously. His lectures were legendary. His writing sparkled with equal brilliance.

The scientific achievements of Knowles led to his becoming a Fellow of the Royal Society, the American Academy of Arts and Sciences, and the American Philosophical Society, a Foreign Associate of the National Academy of Sciences, and a trustee of the Howard Hughes Medical Institute. He was awarded the Charmian Medal, the Bader Award, the Repligen Award, the Prelog Medal, the Robert A. Welch Award in Chemistry, the Nakanishi Prize, and the Davy Medal of the Royal Society. He was an honorary fellow of Balliol College and Wadham College, Oxford, and a recipient of honorary degrees from the University of Edinburgh and the Eidgenössische Technische Hochschule in Zürich. He was appointed Commander of the Order of the British Empire in the Queen's Birthday Honors of 1993.

In 1991, Knowles accepted an offer to become the Dean of the Faculty of Arts and Sciences at Harvard, one that he had declined in 1983. Soon thereafter, Jeremy Knowles closed his research laboratory. Nonetheless, chemical biology has continued to flourish within the Harvard chemistry department. As Dean, Knowles oversaw the formal demarcation of this expanded horizon with the changing of the unit's name to the "Department of Chemistry and Chemical Biology".

Knowles devoted his prodigious skills to the betterment of Harvard University until his death in Cambridge, Massachusetts, from prostate cancer on 3 April 2008. He is survived by his wife, Jane, their three sons, Sebastian, Julius, and Timothy, and seven grandchildren. A memorial service will be held on Friday, 30 May 2008 at 11 am in Memorial Church on the Harvard campus.

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