NATIONAL ACADEMY OF SCIENCES

EMIL THOMAS KAISER

1938—1988

A Biographical Memoir by

F. H. WESTHEIMER

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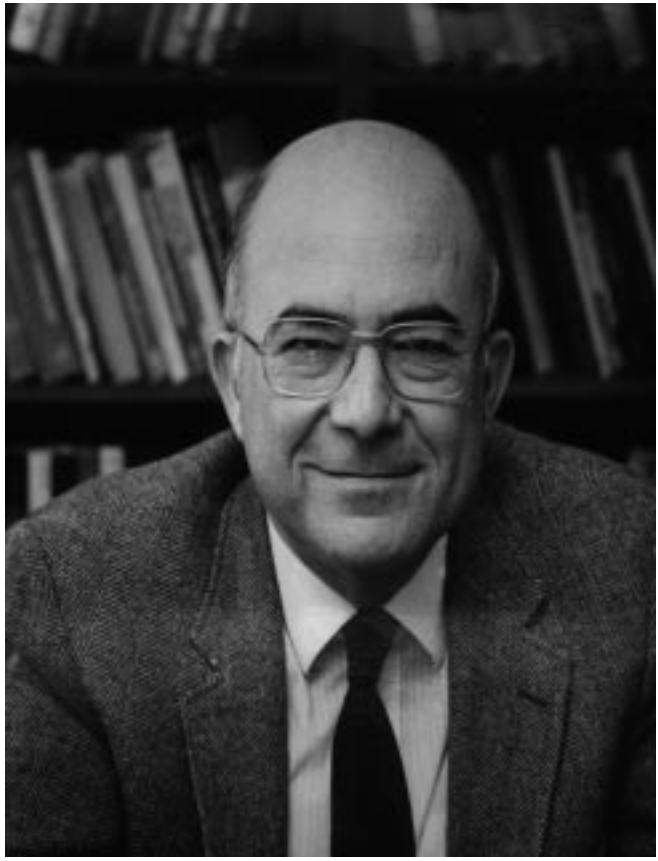

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EMIL THOMAS KAISER

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BY F. H. WESTHEIMER

HUGH WALPOLE'S NOVEL *FORTITUDE* begins with the line, "'Tisn't life that matters! 'Tis the courage you bring to it." That may be true for most people, but it certainly isn't true for the tiny fraction of humanity who are highly creative; their lives matter even more than their courage because they change the world and the lives of others. Tom Kaiser was one of those creative people who changed the world—in his case, the world of science. But even if we were to measure him by Walpole's criterion, he would stand out; his courage was as great as his creativity.

EARLY YEARS

Emil Thomas Kaiser—Tom to his friends—was born in Hungary in 1938. His parents, both of whom were Ph.D. chemists, brought him, when he was an infant, to Canada, where his father had taken a job as a pharmaceutical chemist. Then, when Tom was two, the family moved to the United States, where his father began a long career with the research department of Armour Pharmaceutical Company.

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Obviously, Tom had chemistry in his blood, and it should come as no surprise that his career got off to an early start. His abilities and energy were apparent from the first. He graduated from the University of Chicago at the age of eighteen, and went to Harvard for his graduate work. He completed research for that degree, related to strain in cyclic sulfate esters,[1] with me in only two years, received his doctorate when he was only twenty-one, and began his independent research career. He decided to carry out postdoctoral research with E. J. Corey and afterward with Myron Bender. He and Professor Corey created a remarkable piece of physical-organic chemistry[2] that demonstrated that sulfone anions can retain their chirality, at least briefly; he and Professor Bender investigated the cinnamoyl intermediates[3] formed in the hydrolysis of cinnamoyl esters by trypsin and chymotrypsin. He was now well launched on his career in bioorganic chemistry, with experience in both physical-organic chemistry and enzymology.

At this time Kaiser accepted an assistant professorship at Washington University in St. Louis. His enormous capacity for productive research immediately became clear, and the University of Chicago offered him an assistant professorship in 1963, when he was twenty-five, and a professorship in 1970, when he was thirty-two. Those were among the department's best decisions.

Although his research at Washington University in St. Louis showed both his enormous productivity and his wide grasp of chemical problems, it was only after he came to Chicago that his startling originality came to the fore; it was here that he began the research that made him known in the scientific community. In 1982 he accepted a professorship at Rockefeller University, where he continued his spectacular research. At about the same time he became an editor of *Bioorganic Chemistry*.

Kaiser made two major contributions to that subject and authored a number of other advances that would have distinguished the career of a lesser scientist. One of his major contributions was the development of semisynthetic enzymes and the other concerned amphiphilic helices.

SEMISYNTHETIC ENZYMES[4,5]

Enzyme kineticists separate binding and catalysis. Chemists have been quite successful in identifying the catalytic residues in enzymes and X-ray crystallographers have been successful in identifying the binding sites of substrates and coenzymes on the surfaces of enzymes. Kaiser devised a scheme for making useful new catalytic activities by combining the binding properties of one enzyme with the catalytic activity of an unrelated coenzyme. In particular, he converted a hydrolytic enzyme into one for oxidation-reduction by attaching a flavin coenzyme at the active site of a peptidase, papain. He utilized the binding properties of the peptidase and the oxidation-reduction properties of the coenzyme to make a new enzyme, a chimera, that would effect oxidation-reduction specifically and stereospecificity. This effort was largely successful, and he thus demonstrated how to go about constructing semisynthetic enzymes for many reactions.

In detail, what he did was to synthesize a modified flavin that was substituted with a bromomethyl, or preferably a bromoacetyl group. He then attached this coenzyme to papain. That enzyme has an essential sulfhydryl group at its active site, and this sulfhydryl group reacts readily with and specifically displaces the bromine from the bromoethyl or bromoacetyl group of a modified flavin.[6,7,8] The reaction accomplishes two purposes. It destroys the active site of the protease and at the same time attaches the flavin in a position adjacent to the binding site of the enzyme. The result-

ing semisynthetic enzyme then serves to catalyze the oxidation of a number of substrates. In particular, the substrates that Kaiser chose, such as N-propyldihydronicotinamide, are related to NADH; they are, however, substituted on the nitrogen atom of the pyridine ring with alkyl groups rather than with the adenine-ribose-pyrophosphate-ribose substituent of NADH. The alkyl groups were chosen to match the specificity of papain, which hydrolyzes esters and amides with hydrophobic substituents. A semisynthetic oxidation-reduction enzyme, a chimera of papain and flavin, would be expected to bond, and thus react with hydrophobic substrates, and so it does. Kaiser's best semisynthetic enzyme and best substrate show an increase in rate by a factor of about 1000 over the corresponding uncatalyzed reaction, and a modest stereoselectivity with respect to the diastereotopic hydrogen atoms in the 4-position of the dihydronicotinamide ring. Although the modified papain is not comparably so efficient as natural enzymes, the work clearly demonstrates a principle and shows how to proceed in making new enzymatic activities.

Similar results can be obtained by attaching bromoacetyl flavins to glyceraldehyde-3-phosphate dehydrogenase. The resulting semisynthetic enzyme attacks NADH rapidly; a similar semisynthetic enzyme can be made from hemoglobin.

SYNTHESIS OF PEPTIDES AND PROTEINS

Another powerful idea with semisynthetic enzymes concerns a highly original way of utilizing thiosubtilisin. This modified enzyme had previously been prepared both by Daniel Koshland, Jr.,[9] and Myron Bender[10] and their collaborators by the chemical substitution of a cystein for the catalytically active serine in subtilisin. The resulting protein is still a protease, but a poor one. Kaiser and his coworkers[11,12] showed how to use this semisynthetic enzyme to couple

large peptides. One can only appreciate what can be accomplished with Kaiser's method by analogy to the field of nucleic acid chemistry. The successful synthesis of many polynucleotides depends on the ability of certain enzymes called ligases to join two polynucleotides of moderate size. No naturally occurring enzyme is effective as a protein ligase, and the lack of a ligase severely limits the synthesis of all but the smallest proteins by chemical methods. The solid-state synthesis of peptides, invented by Bruce Merrifield,[13] works beautifully for peptides composed of 20-50 amino acids, but less well for longer ones and has not so far been successfully employed for proteins larger than ribonuclease.

Kaiser and his coworkers showed how to use thiosubtilisin[14] to ligate (that is, to join) activated but unprotected peptides. The originality of this work lies in the appreciation of the utility of having a *poor* enzyme, rather than a good one (i.e., in capitalizing on the fact that the modified enzyme is *inefficient*). Thiosubtilisin reacts rapidly with an activated peptide to form an acylated enzyme and transfers the peptide residue to another peptide, completing the ligation reaction; but, since it is a poor peptidase, it does not attack the resulting product at all rapidly. Because of this work chemical synthesis can now complement the methods of molecular biology in forming proteins. Kaiser's premature death prevented him from exploiting his new methodology; it will remain for others to demonstrate the power of this invention.

In addition to this method for ligation, Kaiser and his coworkers invented a new resin, whereby the amino acids and peptides are built onto an oxime group, that supplements the resins introduced by Merrifield.[15]

AMPHIPHILIC PEPTIDES

Kaiser's opening with respect to amphiphilic proteins was even more important than his invention of semisynthetic enzymes and probably constitutes his major achievement. It offers an important breakthrough in the chemistry of proteins and effectively immortalizes him. Although the scientific community has some understanding of the way in which proteins work, both with respect to binding and catalysis, we are just beginning to understand the reasons for their secondary and tertiary structures. Kaiser's work showed the importance of secondary structure and in particular the reasons why amphiphilic helices are essential to biological activity. One face of an amphiphilic helix is hydrophilic and one hydrophobic; such a helix can lie down on a membrane with the hydrophobic side buried in the membrane and the hydrophilic side facing out to the aqueous solvent. The idea of amphiphilic helices was introduced in 1974 by Segrist et al.[16] based on the helical wheel of Schiffer and Edmundson.[17] But the concept was bare, a description perhaps, but without real substance until Kaiser and his coworkers synthesized peptides that demonstrated the importance of the idea.[18]

They took the naked hypothesis and clothed it. Fitch[19] and McLachlan[20] had previously and independently proposed that apolipoprotein-A, a protein 143 amino acids long, consisted of repeating units 22 amino acids in length in which each unit consisted of an amphiphilic helix. Kaiser and his coworkers[21] verified the hypothesis by synthesizing a peptide of 22 amino acids with minimum homology with the sequence of any of the repeating helical segments of apolipoprotein-A, but which nevertheless shares its biological properties, including in particular the activation of the enzyme lecithin-cholesterol acyltransferase. The synthesis

demonstrated that the activity of apolipoprotein-A does not rest in its detailed sequence, but merely in its secondary structure (that is, in the amphiphilic nature of its repeated helices).

Melittin (that is, bee venom) is a peptide of 26 amino acids. Its toxicity depends on its ability to lyse erythrocytes. The structure shows a cluster of basic amino acids at the C-terminus and an N-terminal sequence of 21 amino acids that has the potential to form an amphiphilic helix. Kaiser postulated that the 4 basic amino acids at the C-terminus acted as a prosthetic, or catalytic group, and should be retained, but that the choice of the other amino acids was nearly arbitrary, provided they form an amphiphilic helix.[22] In collaboration with DeGrado and Kezdy he synthesized a polypeptide 26 amino acids long with the same 4 basic amino acids near the carboxyl terminus as those of melittin. The rest of the sequence was deliberately constructed to diverge widely from the natural. It was designed with 12 leucine residues and 1 tryptophane, properly spaced to mimic the hydrophobic portion of the amphiphilic helix, and 4 serine plus 3 glutamate residues for the hydrophilic portion. The result was a peptide with the properties of bee venom. In particular, it was even more active than the natural venom in the lysis of erythrocytes.

Similar spectacular results were achieved with peptides to mimic the action of calcitonin,[23] corticotropin-releasing factor[24] and endorphins.[25] In each case the mimic served the biological function of the natural product. Synthesis proved that in each case the essential feature of the sequence was an amphiphilic helix with a small prosthetic group in some cases; provided these features were preserved, the precise sequence was irrelevant. W. DeGrado has carried this idea to its logical extreme, imitating ion channels with helices composed of only two kinds of amino acids, leucine and

glutamic acid, and ignoring natural sequences almost completely.[26]

The results are important not only with respect to the concept of amphiphilic helices, but also with respect to protein chemistry in general. The same enzyme in different species is represented by proteins that boast only partial homology. This is necessarily so; life would never have developed if only one combination and permutation of amino acids could serve a given function. A protein with only 100 amino acid residues—10 each of 10 different kinds—would allow $2x10^{92}$ permutations, an incredible number far beyond imagination. There has not been anywhere near enough time to examine even a billionth of a billionth of a billionth of these possibilities; after all, Earth is only 10^{17} seconds old. The living world can exist only because an enormous number—even if it is only a tiny fraction of the permutations for a protein—can carry out its essential function perfectly well. Here in Kaiser's work is part of the experimental demonstration that this is so.

SITE-DIRECTED MUTAGENESIS

Although semisynthetic enzymes and amphiphilic helices constitute the principal contributions from Kaiser's laboratory, at least one other aspect of his work (i.e., site-directed mutagenesis) demands attention. This technique is common today; it was relatively new in 1987. Kaiser and his coworkers substituted phenylalanine for tyrosine at position 198 in carboxypeptidase.[27] The hydroxyl group of the latter residue had been assigned by others to an important role in enzymatic catalysis. But the mutant enzyme that Kaiser and his coworkers created with phenylalanine in place of tyrosine, works perfectly well, and the mechanism of action of carboxypeptidase had therefore to be revised. Here

in Kaiser's work is an early example of the power of site-directed mutagenesis in enzymology.

SCIENTIFIC SIGNIFICANCE

We have described Kaiser's work, but we have just begun the task of evaluating it. What he did really was to introduce a new field into chemistry. We (the bioorganic chemists) have been trying for some time to understand the action of enzymes and other active biological molecules. An enormous effort has been expended in our attempts to make enzyme models on the grounds that we cannot claim really to understand how enzymes work until we can build our own. We have had indifferent success so far in this undertaking; progress has been modest relative to the effort involved.

Kaiser introduced a different approach. If enzymes—most enzymes, at any rate—and receptors are proteins, we should then understand the way proteins interact with their receptors and membranes; we should at least understand the importance of secondary structures.

COURAGE

We have described the remarkable science Kaiser achieved and outlined the impact of his work on the future of chemistry, but we have told only half the story. The other half takes us back to Hugh Walpole and to courage. Before paying tribute to Kaiser's courage, let me say a bit about him as a person. He was friendly and smiled easily and often. He was devoted to his wife Bonnie and their children; admired his parents; and valued his graduate students. He was perceived as fair. He had no disputes other than friendly scientific ones. People trusted him; there was never a doubt that he would protect information given to him in confidence or give credit where credit was due.

When he became ill and his kidney problem had been diagnosed, he scarcely slowed up. His kidneys failed completely, and to clear his system of urea he had to undergo dialysis three times a week for four hours at a time. He wrote in 1988 that, "I have managed to utilize the time during my treatment for reading, but there is no question that having to go for dialysis on a regular basis is quite confining." Quite confining—no more complaint that that. It is hard to imagine facing such an ordeal with that much raw courage. He continued all his activities. He wrote, "Otherwise, everything is going well. I was able to travel to give seminars during the Fall [these included a lecture in Milan, named lectures at Virginia and North Carolina, the Calvin lectures at Berkeley, and more] since I could arrange to be treated . . . ." In other words, dialysis could be arranged all over the world; he did not allow anything—certainly not personal discomfort or risk—to interfere with his contributions to science. Tom Kaiser made these matter-of-fact comments concerning dialysis and then went on to discuss his discoveries. One can only shake one's head in awe. Kaiser's life during the year of dialysis would have stopped almost everyone; he faced it with so much courage that his life went on almost normally.

During the many months that he underwent dialysis three times a week, he talked about a kidney transplant. He was always upbeat, always optimistic. He quoted the favorable statistics on the operation—better than 90% successful—and felt absolutely confident that he would be one of the majority. His spirit—his smile and ebullient optimism—were contagious. Those who knew him were convinced by his enthusiasm that the operation would be a great success.

Kaiser was elected to the National Academy of Sciences in 1987, and he had planned to attend the meeting in April 1988 in order to sign the book and be formally inducted

into the Academy. At the meeting of the Section of Chemistry we learned that he would not attend, but we were happy about the reason: a proper kidney had been found for him, and he would receive a transplant in Boston while the Academy met in Washington. The operation appeared to be a great success; he and the doctors were delighted. A few months later our optimism, and Tom, were gone.

But we can still be buoyed by that optimism. His friends will want to face any crisis in their own lives with half the courage he displayed in his. Science has profited by the openings he made; we—his friends and scientific heirs—can try to carry on in the pathways he pioneered. But we need, too, to hail his courage and will to overcome personal obstacles. We can only speculate on what he would have accomplished if he had survived. He was enormously productive, enthusiastic, and full of new ideas. We know that we have lost a friend, that the scientific community has lost a great scientist, and we have all lost a role model for facing adversity with courage.

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