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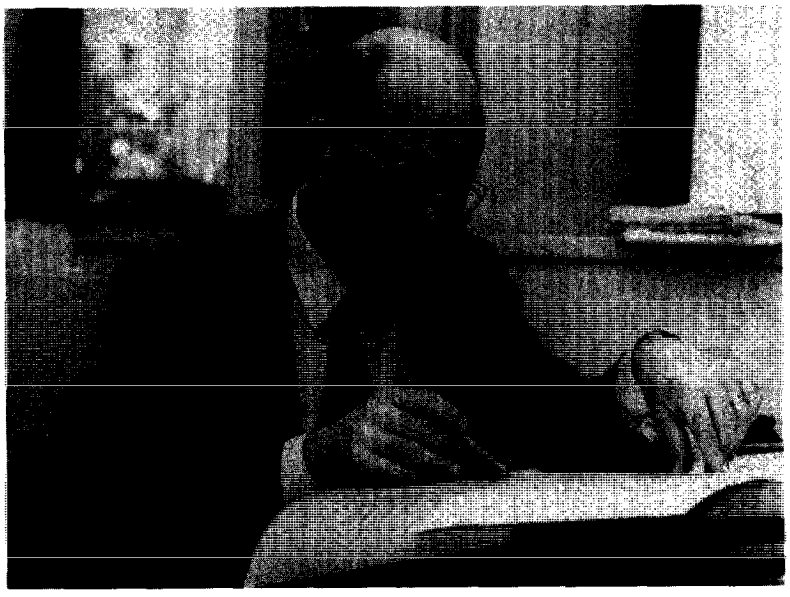
# IN MEMORIAM

## Emil Thomas Kaiser
## (February 15, 1938–July 18, 1988)



*Introduction.* 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 emphatically is not 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 in so doing change the lives of others. Tom Kaiser was one of those creative people who change the world—in his case, the world of science. But even if we were to measure him and his life by Walpole's criterion, Tom Kaiser 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 position as a pharmaceutical chemist. Then, when Tom was 2, the family came to the United States, where his father began a long career with the research department of the Armour Pharmaceutical Co. Obviously, Tom Kaiser 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 was graduated from the University of



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Chicago at the age of 18, and came to Harvard for his graduate work. It was my good fortune that he chose to work with me for his Ph.D. He completed his research problem (*1*), related to strain in cyclic sulfate esters, in only 2 years, received his doctorate when he was only 21, 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 25, and a Professorship in 1970, when he was 32. These 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 famous in the scientific community. Five years ago, he accepted a Professorship at the Rockefeller University, where he continued his spectacular research. At about the same time he became a member of the Editorial Board of this journal.

Kaiser made two major contributions to bioorganic chemistry, and a number of other advances that would have distinguished the careers of lesser scientists. 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 the 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 stereospecifically. 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. Papain has an essential sulfhydryl group at its active site, and this sulfhydryl group reacts readily and specifically to displace the bromine from the bromomethyl or bromoacetyl group of a modified flavin (*6–8*). 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 resulting semisynthetic enzyme then serves to catalyze the oxidation of a number of substrates. In particular, the substrates Kaiser chose, such as *N*-propyldihydronicotinamide, were related to NADH; they were, however, substituted on the nitrogen of the pyridine ring with alkyl groups rather than with the ribose–pyrophosphate–ribose–adenine substituent of NADH. The alkyl groups were chosen to match the specificity of papain, which hydrolyzes esters and amides with hydrophobic substituents. The semisynthetic oxidation–reduction enzyme, the chimera of papain and flavin, would be expected to bind, and thus react with, hydrophobic substrates, and so it does. The best semisynthetic enzyme and the best substrate show an increase in rate by about a factor of 1000 over the uncatalyzed reaction, and a modest stereoselectivity with respect to the diastereotopic hydrogen atoms in the 4-position of the dihydronicotinamide ring. Although this 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 bromoacetylflavins 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 thiolsubtilisin. This modified enzyme had previously been prepared by both Daniel Koshland, Jr. (*9*), and Myron Bender (*10*) and their collaborators by the chemical substitution of a cysteine residue for the catalytically active serine in subtilisin. The resultant protein is still a protease, although a poor one. Kaiser and his co-workers (*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 such a ligase severely limits attempts to prepare all but the smallest proteins by chemical methods. The solid-state synthesis of peptides, invented by Bruce Merrifield, works beautifully for peptides composed of 20 amino acids, but less well for much longer ones, and has so far not been successfully exploited for proteins larger than ribonuclease.

Kaiser and his co-workers showed how to use thiosubtilisin 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, the chemical synthesis of proteins can now complement and compete with synthesis by the methods of molecular biology. 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 co-workers invented a

new resin, where the amino acids and peptides are built onto an oxime group, that supplements those invented by Merrifield (*13, 14*).

*Amphiphilic peptides.* Kaiser's opening with respect to amphiphilic proteins was even more important than his invention of semisynthetic enzymes and constitutes, in the view of this reviewer, his major achievement. It offers an important breakthrough in the chemistry of proteins and effectively imortalizes him. Although the scientific community has some understanding of how enzymes work, both with respect to binding and with respect to catalysis, we have very little understanding of 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 solvent. The idea of amphiphilic helices was introduced in 1974 by Segrest *et al.* (*15*) based on the "helical wheel" of Schiffer and Edmundson (*16*). But the concept was bare, a description, perhaps, but without real substance until Kaiser and his co-workers synthesized peptides that demonstrated the importance of the idea (*17*).

They took the naked hypothesis and clothed it. Fitch (*18*) and McLachlan (*19*) had previously and independently proposed that apolipoprotein-A, a protein 243 amino acids long, consists of repeating units, 22 amino acids in length, where each unit consists of an amphiphilic helix. Kaiser and his co-workers (*20*) verified the hypothesis by synthesizing a peptide of 22 amino acids with minimal homology with the sequence of any of the repeating helical segments of apo-A, but which nevertheless shared 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 decided that the four basic amino acids at the C-terminus acted as a prosthetic, or catalytic group, and should be retained, but that the choice of other amino acids was nearly arbitrary, provided that they form an amphiphilic helix (*21*). 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 constructed so as 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 (*22*), corticotropin-releasing factor (*23*), and endorphins (*24*). 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, in some cases, a small prosthetic group; provided that these were preserved, the precise sequence was irrelevant. W. DeGrado has carried this idea from Kaiser's laboratory to its logical extreme, imitating ion channels with helices composed of only two amino acids, leucine and glutamic acid, and ignoring natural sequences almost completely (*25*).

The result is important not only with respect to the concept of amphiphilic helices, but also with respect to protein chemistry in general. Many sequences, with only partial homology, represent the same enzyme in different species. 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 acids, with 10 each of 10 different kinds, would allow $2 \times 10^{92}$ 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 those possibilities—after all, the earth is only $10^{17}$ s old. The living world can only exist because an enormous number, even if it is only a tiny fraction of the permutations possible for any 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 proteins constitute the principal scientific contributions from Kaiser's laboratory, at least one other aspect of his work, i.e., site-directed mutagenesis, demands attention. In particular, Kaiser and his co-workers substituted phenylalanine for tyrosine at position 198 in carboxypeptidase (*26*). The phenolic hydroxyl group of the tyrosine residue had been assigned by others an important role in enzymatic catalysis. But the mutant enzyme that Kaiser and his co-workers created, with phenylalanine in place of tyrosine in carboxypeptidase, works perfectly well. The mechanism of action of carboxypeptidase must necessarily be revised. Here is a perfect example of the use of site-directed mutagenesis to obtain an important result in enzymology.

*Scientific significance.* When we have described Kaiser's work, we have just begun the task of evaluating it, because what he did was to introduce what is really a new field of 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 ground 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 rather 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 in which proteins interact with their receptors and with membranes; we should at least understand the importance of their secondary structures.

After we have described the remarkable science that Kaiser achieved, and

when we have outlined the impact of his work on the future of chemistry, we have told only half the story; the other half brings us back to Hugh Walpole and to courage. Before, however, 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 loved Bonnie, was devoted to his children, admired his father, and valued his graduate students. He was fair and 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, and that he would give credit where credit was due.

When he became ill, when his kidney problem had been diagnosed, he scarcely slowed up. His kidneys failed completely, and he had to undergo dialysis three times a week for 4 hours at a time to clear his system of urea. He wrote 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 than 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 the advance of science. Tom Kaiser made these matter-of-fact comments concerning his dialysis and then went on to discuss his discoveries. One can only shake his head in awe and recall Walpole's phrase about courage. 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 ebulient optimism, and his smile were contagious; those who knew him were convinced by his enthusiasm that the operation would be a great success.

Kaiser had been elected to the National Academy of Sciences and had planned to attend the meeting last April, in order to sign the book as he was inducted into the Academy. At the meeting of the Chemistry section last spring, we learned that he would not attend, but were happy with the reason: A proper kidney had been found for him, and he would receive the 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 try to face any crisis in their own lives with half the courage he displayed in his. Science has profited by the opening he made; we, his friends and scientific heirs, can try to carry on in the pathways he pioneered. But we need, too, to hail and imitate his courage and his will to overcome his personal obstacles. We can only speculate what he would have accomplished if he had survived. He was enormously productive, enormously enthusiastic , and full of new ideas. We only know that we have

lost a friend, the scientific community has lost a great scientist, and we have all lost a role model for facing adversity with courage.

## REFERENCES


1. KAISER, E. T., AND WESTHEIMER, F. H. (1963) *J. Amer. Chem. Soc.* **85,** 602.
2. COREY, E. J., AND KAISER, E. T. (1961) *J. Amer. Chem. Soc.* **83,** 490.
3. BENDER, M., AND KAISER, E. T. (1962) *J. Amer. Chem. Soc.* **84,** 2256.
4. HILBERT, D., AND KAISER, E. T. (1987) *Biotechnol. Genet. Eng. Rev.* **5,** 297.
5. KAISER, E. T. (1987) *Ann. N.Y. Acad. Sci.* **501,** 14.
6. LEVINE, H. L., NAKAGAMA, Y., AND KAISER, E. T. (1977) *Bichem. Biophys. Res. Commun.* **76,** 64.
7. LEVINE, H. L., AND KAISER, E. T. (1978) *J. Amer. Chem. Soc.* **100,** 7670.
8. LEVINE, H. L., AND KAISER, E. T. (1980) *J. Amer. Chem. Soc.* **102,** 343.
9. NEET, K. E., AND KOSHLAND, D. E., JR. (1966) *Proc. Natl. Sci. USA* **56,** 1606.
10. POLGAR, L., AND BENDER, M. L. (1966) *J. Amer. Chem. Soc.* **88,** 3153.
11. NAKASUKA, T., SASAKI, T., AND KAISER, E. T. (1987) *J. Amer. Chem. Soc.* **109,** 3808.
12. KAISER, E. T. (1988) *Angew. Chem.* **27,** 913.
13. DEGRADO, W. F., AND KAISER, E. T. (1980) *J. Org. Chem.* **45,** 1295.
14. NAKAGAWA, S. H., AND KAISER, E. T. (1983) *J. Org. Chem.* **48,** 678.
15. SEGREST, J. P., JACKSON, R. L., MORRESETT, J. D., AND GOTT, A. M., JR. (1974) *FEBS Lett.* **38,** 247.
16. SCHIFFER, M., AND EDMUNDSON, A. B., (1967) *Biophys. J.* **7,** 121.
17. KAISER, E. T., AND KEZDY, F. J. (1984) *Science* **223,** 249.
18. FITCH, W. M. (1977) *Genetics* **86,** 623.
19. MCLACHLAN, A. D. (1977) *Nature (London)* **267,** 465.
20. YOKOYAMA, S., FUKUSHIMA, D., KAISER, E. T., AND KEZDY, F. J. (1980) *J. Biol. Chem.* **255,** 7333.
21. DEGRADO, W. F., MUSSO, G. F., LIEBER, M., KAISER, E. T., AND KEZDY, F. J. (1982) *Biophys. J.* **37,** 329.
22. MOE, G. R., MILLER, R. J., AND KAISER, E. T. (1983) *J. Amer. Chem. Soc.* **105,** 4100.
23. LAU, S. H., RIVIER, J., VALEE, W., KAISER, E. T., AND KEZDY, F. J. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3070.
24. TAYLOR, J. W., MILLER, R. J., AND KAISER, E. T. (1983) *J. Biol. Chem.* **258,** 4464.
25. LEAR, J. D., WASSERMAN, Z. R., AND DEGRADO, W. (1988) *Science* **240,** 1177.
26. GARDELL, S. J., HILVERT, D., BARNETT, J., KAISER, E. T., AND RUTTER, W. J. (1987) *J. Biol. Chem.* **262,** 567.


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