Fifty Years Ago

The identification of 'active acetate' as acetyl-CoA

For many years we have come to regard the reactions of the biological degradation, synthesis and transformation of fatty acids as 'contained' in the kinetic and thermodynamic reactivity of thioesters. But it has not always been so. 'Active acetate' had long been thought to be most likely acetyl-phosphate. It was in 1951 that Feodor ('Fitzi') Lynen's group reported that 'active acetate' was the thioester of coenzyme A (Lynen, F., Reichert, E. and Rueff, L., Ann. Chem. 574 (1951) 1–32). The history of this discovery is recounted most vividly by Lynen himself in the paragraphs that follow, which are taken from an autobiographic chapter that appeared a few years ago (F. Lynen, in: Slater, E.C., Jaenicke, R. and Semenza, G. (Eds.), Comprehensive Biochemistry, Vol. 38; Personal Recollections Vol. IV; Elsevier, Amsterdam, 1995; pp. 1–19).

Giorgio Semenza
Former Managing Editor

... At this stage, which was reached in 1941, my work remained stagnant for many years. Hitler had attacked Russia, and the bomb attacks on our country were so intensified that research in our institute became more and more difficult. In December, 1944, the Chemical Institute in Munich was completely destroyed. Fortunately, a few months before, I had moved my laboratory equipment to a place outside Munich and found a temporary room in the Botanical Institute. It happened that at that time we again got access to foreign literature after many years of isolation. Only then did I become informed about the progress made in the meantime. 'Active acetate' was now recognized in many laboratories as occupying a central position, not only in carbohydrate catabolism but also in lipid metabolism. Lipmann had discovered coenzyme A, and there was experimental evidence that 'active acetate' was a derivative of this coenzyme. I was very eager to embark immediately on experiments with coenzyme A, but pigeon livers were required for the experiments, and at this time no pigeons could be found because they were being eaten and most of them were selling for high prices on the black market. Only after the revaluation of the German currency could I start on these experiments with the assistance of Ernestine Reichert. This led in 1950 to the identification of 'active acetate' with the thioester between acetate and coenzyme A.

I remember in every detail how this discovery came about. My brother-in-law, Theodor Wieland, was on holiday in his parents' house next to ours. He had worked in Richard Kuhn's laboratory on pantothenic acid, the vitamin discovered by Lipmann to be a constituent of coenzyme A. We spent a whole night in shoptalk about the possible link between acetate and pantothenic acid but could not come to any conclusion. On my short way back to our garden it suddenly came to my mind that the acetyl residue might be bound, not to pantothenic acid at all, but to sulfur. I recalled that in Lipmann's last paper on the composition of the purified coenzyme A preparations he had mentioned the presence of sulfur, but he did not pay much attention to it because his preparations were not yet pure. In addition, it was known that all enzymatic reactions studied in which coenzyme A was involved required the addition of glutathione or cysteine, presumably as agents for the binding of inhibitory heavy metals. Third, as a chemist I knew that sulfhydryl groups are more acidic than hydroxyl groups, which means that acetic acid bound to sulfur must have the properties of an acid anhydride and this should have the capacity to acetylate amines or alcohols. The crucial step for me was that I put the three things together. I became very excited, hurried into my study, and looked into *Beilstein*. Soon I found that thioacetic acid was known to react with aniline to form acetanilide, I thus became completely convinced that 'active acetate' must be a thioester. Ernestine Reichert was very surprised when I walked into the laboratory next morning and told her that we would now embark on the isolation of acetyl CoA from yeast Kochsaft. In two months' time this goal was reached, and by comparison with a synthetic thioester which was given to me by an organic chemist of the Munich laboratory, who happened to be working with such compounds, it could be proved that my prediction was correct.

This whole story was most exciting, but it was to become even more dramatic when my short publication was sent to *Angewandte Chemie* to be published in the December issue. Everything now seemed so simple to me that I could hardly believe that nobody else in the meantime could have had the same idea. Every day I expected a publication of this kind, and I was relieved only when I heard by letter from Otto Meyerhof and Carl Neuberg that my paper had come to the biochemists in the United States like a bombshell. I realized only later that most of the biochemists were prejudiced by the idea that active acetate could only be some kind of phosphate derivative. With the discovery of the thioester bond in acetyl CoA, the energy-rich phosphate bond had lost its unique role in metabolic reactions, and it was to be expected that still other energy-rich bonds would be discovered.

I mention this story in such detail because it illustrates how discoveries can happen. I had worked or thought continuously for thirteen years on the acetate problem and was relieved only when it was solved. As already pointed out, I have the philosophy that persistence is an essential element in science, which, however, should not exclude tackling more than one problem at a time. Also, a scientist wants to be happy, and if he does not progress with one problem, he might be more successful with others. ...

The elucidation of the structure of acetyl CoA resulted in an invitation to attend a Gordon Conference in 1951. Everything was arranged, but, unfortunately, shortly before the conference I again broke my leg in skiing and again had to spend several weeks in a cast. This provided me with the time

0014-5793/01/$20.00
PII: S0014-5793(01)03198-2

necessary to think about new research projects. It was not at all difficult to find them. Once the structure of acetyl CoA was worked out, a detailed chemical scheme of fatty-acid oxidation could be written. I presented it in my first papers and called it the 'fatty acid cycle.' The next step then was to isolate and identify the four enzymatic components participating in this process. Here again my training as a chemist was a big help. The intermediates in the oxidative reaction are α,β-unsaturated-, β-hydroxy-, and β-ketocarboxylic acids bound to coenzyme A. In order to learn something about their chemical properties, Luise Wessely and Werner Seubert synthesized simple models in which these carboxylic adds were bound, not to coenzyme A, but to N-acetyl cysteamine. It turned out that some of these thioesters possess characteristic ultraviolet-absorption bands, and it seemed possible to use these properties in specific enzyme assays. When we started work on the fatty-acid oxidation enzymes, only acetyl CoA was available, and it had to be isolated from yeast Kochsaft. Thus the only reaction step which we could study in the beginning was the reversible condensation of two acetyl CoA's to form acetoacetyl CoA. Luise Wessely tried very hard to demonstrate this reaction in experiments with liver extracts, using the absorption band of acetoacetyl CoA at 300 mμ. This band appears only at an alkaline pH because it is related to the formation of the enolate ion. For that reason she worked at pH 8. But even under these conditions, and though we spent much of our very valuable acetyl CoA from yeast, the absorption band did not appear. We were all very depressed, especially since I was supposed to go to the International Biochemistry Congress in Paris and would have liked to present new results. When I at last left the laboratory to go to Paris, in some kind of despair I turned to my other brother-in-law, Otto Wieland, who at that time worked with me, and he asked me what he could do during my absence. I said, 'I am sure it will not work, but why don't you see whether our model acetoacetyl-N-acetyl cysteamine can react with DPNH or TPNH in presence of liver extracts?' How great was my surprise and excitement when I heard after my return from Paris that this experiment had worked! By the use of this simple assay, the purification of β-hydroxyacyl CoA dehydrogenase, and soon also the demonstration of the enzyme thiolase, became possible. Once we knew that thiolase was rather active in liver extracts, Luise Wessely repeated the previous experiments, working now at pH 9 instead of pH 8. To our great astonishment the absorption band at 300 mμ now appeared, because at the more alkaline pH the thiolase reaction is shifted to the side of condensation. Thus I learned a new lesson for a scientist – be naïve and try an experiment, even if the chances for its success are very small. I have the feeling that many of my colleagues in biochemistry – and the same may be true also for other fields of science – often spend more time in discussing the pros and cons of an experiment than it would take to perform it. I think that a good many opportunities for new discoveries are missed that way.

Note from the Editorial Office:

We would like to occasionally remind the readers of *FEBS Letters* of important events in the history of biochemistry. Thus, we will mark such anniversaries by printing some information related to the people involved in these discoveries. We hope that you enjoy these recollections.