This essay reviews some of the types of problems under investigation during the period of 1940–1950 and the influence two exceptional scientists would have on my early development as a biochemist. To put my life into some kind of perspective in regard to this particular period, I should provide some data about my early academic situation. I started my graduate studies with a Master's degree from the Department of Biological Chemistry at the University of Michigan, whose Chairman was Howard B. Lewis. He was a dynamic lecturer and one of the pioneers of research in intermediary metabolism. For my Doctorate degree I moved to Harvard Medical School to work with A. Baird Hastings (see Fig. 3). Both Lewis and Hastings were principal mentors during my career. I then spent 10 years on the faculty of the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania in Philadelphia with a 2-year sabbatical period at the Medical Nobel Institute with Hugo Theorell. Eventually I returned to the Boston area as Head of the Biochemistry Program in the Department of Biology, Massachusetts Institute of Technology.

These features of my academic life establish the setting from which I would view the imprint that my seniors, for example, Krebs (Fig. 1) and Lipmann (Fig. 2), would make on my chosen field of intermediary metabolism. The whole approach to biochemical research had a monumental advance with the availability of radioactive or stable isotopes of carbon. In 1939 Hastings (Fig. 3) had been able to arrange for the production of $^{11}$C by our cyclotron in Cambridge. Its very short half-life, however, limited the types of problems that could be finished within a 4–5-h time limit. Despite this, a team of organic chemists and biochemists was able to synthesize two forms of lactic acid, one labeled on the carboxyl carbon and the other on the $\alpha$ or $\beta$ carbons (2), feed either one to fasted rats, and then isolate and measure the radioactivity of the liver and muscle glycogen. The initial goal was to determine whether these experiments confirmed the validity of Schoenheimer's proposal (3) of the Dynamic State of Body Constituents. Indeed, lactic acid did contribute to the pool of glycogen precursors. However, a new finding did emerge, namely, in its conversion to liver glycogen the carboxyl carbon of lactic acid is replaced in part with carbon originating from carbon dioxide (4, 5). Research on the utilization of carbon dioxide as a substrate of reactions had been pioneered by Harland Wood and Chester Werkman (7, 8) in microbial systems, but the existence of this reaction in higher systems had yet to be verified. The Wood-Werkman reaction involves the carboxylation of pyruvate to yield oxalacetate.

\[
\text{CH}_3\text{COCOOH} + \text{CO}_2 \leftrightarrow \text{HOOCCH}_2\text{COCOOH}
\]

\[
\text{REACTION 1}
\]

Pyruvate is formed by the oxidation of lactic acid (\(\text{CH}_3\text{CHOHCOOH}\)).

Earl Evans (9) of the University of Chicago had spent a sabbatical year in Krebs’ laboratory in England working on reactions of a cycle of reactions that had been proposed by Krebs (10,
for the oxidation of pyruvate by its condensation with oxalacetate to yield citric acid. Then, through a series of reactions involving cisaconitic acid, isocitric acid, α-ketoglutaric acid, and succinic, fumaric, and malic acids, oxalacetic acid is regenerated. Thus, any of the aforementioned acids could behave catalytically in the cycle because the end product of the reaction, oxalacetate, would be available to react with pyruvate for another round of the cycle. Krebs initially proposed the involvement of a seven-carbon compound, oxalcitraconic acid, which by oxidative decarboxylation would yield the six-carbon citric acid. As will be seen, a better proposal would emerge when it was realized that pyruvate was not the actual reactant in the initial condensation with oxalacetate but rather a two-carbon compound produced by the oxidative decarboxylation of pyruvate. However, the path to this conclusion did not emerge easily.

Liver tissue differs from muscle in a major way in that oxalacetate can be formed directly from pyruvate and carbon dioxide by the Wood-Werkman reaction, and hence the addition of an organic acid of the cycle would not be required in this tissue. Aware of this finding, Krebs had written to Hastings about coming to Harvard to test this proposed role of carbon dioxide with radioactive $^{13}$C. However, this visit proved impossible in 1940 because of England’s serious involvement in World War II. However, Evans and Slotin (12), using pigeon liver minces, were able to conduct the experiment in Chicago and reported that α-ketoglutarate was labeled only in the carboxyl group proximal to the keto group. This finding appeared to require a restatement of the cycle with cisacmonitate being the product of the reaction of pyruvate and oxalacetate because citric acid is a symmetrical compound, and the isotope should therefore be expected to appear in either carboxyl group of α-ketoglutarate to an equal extent.
This paradox was temporarily resolved by a theory proposed by Ogston (13) that even though citric acid appeared to be symmetrical, in fact it reacts asymmetrically with an enzyme if the latter binds to its substrate with a three-point attachment. Thus, under these circumstances citric acid could remain in the cycle. However, from recent work in Koshland’s laboratory on isocitrate dehydrogenase they recognized fallacies in hidden assumptions of Ogston’s hypothesis. They reported that the three-point attachment model could not be the reason for the distinction. In fact, they found that “the major difference was the orientation of the fourth group or the hydroxyl group of citric acid” (14).

However, this hypothesis did not arrive in time to keep us from making a similar erroneous conclusion in some research we had commenced at the University of Pennsylvania. At Pennsylvania we had the opportunity of working with the stable isotope of $^{13}$C, where time was not a factor in our experiments. A team composed of Samuel Gurin, Warwick Sakami, D. W.
Wilson, and myself decided to tackle the problem of how fatty acids and ketone bodies are oxidized, possibly through the Krebs cycle (15), a proposal that had been advocated previously by others (16, 17). A pressing problem at that time was the question of whether fatty acids could be converted to carbohydrates. I think that this problem arose because diabetic patients oxidized fats when the metabolism of carbohydrates was prevented by a lack of insulin.

Our experimental tissues were homogenates of guinea pig or rabbit kidneys. To these preparations we added carboxyl labeled acetate or carboxyl and carbonyl doubly labeled acetoacetate together with banks of unlabeled members of the citric acid cycle, principally \( \alpha \)-ketoglutarate. At the end of the incubation we isolated the residual \( \alpha \)-ketoglutarate and its oxidation products, succinic and fumaric acids. In the case of \( \alpha \)-ketoglutarate we found that the isotopic carbon was present entirely in the carboxyl carbon distal to the keto group. Thinking that our experiments supported those of Evans, we also published our belief that citric acid was not a member of the tricarboxylic acid cycle. Of course, the Ogston hypothesis, which was reported soon after our publication, invalidated our conclusion.

However, our results did provide an explanation of our principal objective of whether fatty acids and ketone bodies are or are not converted to carbohydrates or carbohydrate precursors. As an acetate molecule is oxidized by the reactions of the citric acid cycle, two molecules of carbon dioxide are produced by one turn of the cycle. However, the two molecules of carbon dioxide did not have their origin in the original acetate. The acetate carbons remained in the
oxalacetate produced in the first cycle. Then, by equilibrium reactions in the carbohydrate metabolic pool, some labeled carbon may be found in glucose intermediates and in glycogenic amino acids such as alanine. However, this evidence does not justify a conclusion that fatty acids are converted to carbohydrates in the conventional sense, because there is no net gain in the amount of carbohydrate precursor in the form of oxalacetate. Only a normal molecule is replaced by a labeled one.

Perhaps the greatest advance in concepts of intermediary metabolism during these years was the eventual realization that a carbohydrate precursor, pyruvate, and a fatty acid representative, acetate or a ketone body, utilize a common oxidative pathway, through the participation of an active derivative of the two-carbon compound, acetate. The identity of this derivative took a long time to emerge. The classic experiments of Medes, Weinhouse, and Floyd (18) had proven decisively that $\beta$-oxidation of a fatty acid occurred with some of the product undergoing oxidation via the tricarboxylic acid (TCA) cycle and the residue condensing into ketone bodies, acetoacetic acid and $\beta$-hydroxybutyric acid. Furthermore, although acetoacetate breaks down into two-carbon units in liver slices in preparation for oxidation, they do not reform acetoacetate with carbon atoms in any different distribution than was originally present (19).

It took a long time for researchers to concur that ketone bodies are not simply dead-end products of fat metabolism, there being evidence both pro and con with the several tissue systems used in experiments. For instance, Krebs and Eggleston (20) reported in one study with heart tissue that all of the acetoacetate disappearing in their experiment could be accounted for by the formation of $\beta$-hydroxybutyric acid. In our kidney homogenates the optimal metabolism of acetoacetic acid was achieved when an acid of the citric acid cycle was present in the incubation, particularly $\alpha$-ketoglutaric acid. As was later discovered, there is a coenzymic function relating the oxidation of $\alpha$-ketoglutaric acid and the activation of acetoacetate for its oxidation (21) (see below).

The conclusion of this essay examines the history of the search for the identity of the active derivative of acetate and how the final form of the citric acid cycle was achieved.

Lipmann had gained his early reputation by his proposal of how chemical energy, stored in polyphosphate compounds such as ATP, provides the energy required for biosynthetic reactions (22). Although his concept of “high energy phosphate” required reinterpretation, it was useful in explaining how compounds could become activated and participate in certain reactions. One such example was the production of acetylphosphate in pyruvate oxidation (23). As attractive as this compound seemed as a participant in certain microbial systems, it was not co-reactive with oxalacetate in the citric acid cycle.

Lipmann then turned to a study of the acetylation of sulfonamides (24), which led him to the discovery of coenzyme A. At first this research seemed to have limited significance. However, this attitude changed rapidly when the composition of purified coenzyme A (25) was established in research with Dr. Beverly Guiard as a derivative of pantothenic acid, a vitamin under investigation at the University of Texas, in the laboratories of Esmond Snell and Roger Williams. Coenzyme A was a cofactor relating the transacetylation reactions (21, 26) of pyruvate and $\alpha$-ketoglutarate oxidation to the activation and oxidation of acetoacetate. Also importantly it was a participant with oxalacetate in the synthesis of citric acid. As a result of the famous paper by Novelli and Lipmann (27) the full significance of this coenzyme was finally realized and the two worlds of Hans Krebs and Fritz Lipmann converged. In 1953 their combined works were recognized by a joint award of the Nobel Prize in Medicine or Physiology. In Boston there was a substantial celebration for Fritz as a prominent and much beloved member of the biochemical and medical faculty of Harvard Medical School.

Both Lipmann and Krebs had been born and educated in Germany. Before World War II both had met and worked together in the laboratory of Otto Meyerhof and Otto Warburg, two of Germany’s most outstanding biologists. Because of the Nazi persecution of Jews, Krebs emigrated to England, and Lipmann as well as Meyerhof emigrated to the United States. Both Krebs and Lipmann established distinguished schools of students in their adopted countries. Lipmann contributed to many other areas of biochemistry, including the reactions of protein synthesis. Krebs in turn had published two important papers that were to influence my life on the biosynthesis of hypoxanthine in pigeon liver slices (28, 29). In these papers methods were reported on the chemical degradation of uric acid to its individual carbon and nitrogen atoms. With the availability of the $^{13}$C and eventually $^{14}$C isotopes, the study of the biosynthesis of
uric acid became possible in 1946 (30–33). This goal was expanded in 1948 by G. Robert Greenberg (then at Western Reserve University) by his evidence that purines are synthesized in pigeon liver homogenates and extracts (34) in the form of their ribonucleotides (35). It has been a source of pleasure to me that our two laboratories have enjoyed mutually cordial relationships over the years. The dual but independent approach to the clarification of the individual steps in this biosynthetic system was essential. Even more satisfying has been my close friendship with my own group of gifted and productive students and postdoctoral fellows.

The story of purine biosynthesis, however, is for another time. This essay has tried to place two great scientists, Hans Krebs and Fritz Lipmann, in the context of the time when their studies on carbohydrate and fat metabolism were of primary interest to the biochemical community. I met Krebs only twice, once when I was in England in 1947 to attend an International Congress of Physiology. The second time was at a reception following the Dunham Lectures at Harvard Medical School. Invited to make some comments on that occasion, I was able to convey to him how much my own scientific endeavors had depended on the background he had provided in the biochemical literature.

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