The Tricarboxylic Acid Cycle

Hans Krebs' nobel prize-winning discovery of a central metabolic process

Steven Benner

As is the case with all history, the goal of history of science is to elucidate facts that aid in the understanding of science. Philosophers have relied heavily on these facts and have developed several more or less formal theories about how scientific theories are formulated and come to be accepted. A theory about theories (or a science of science) of course creates numerous paradoxes. For example, it is not clear how such a theory would ever be confirmed. We will handle this and other paradoxes of similar nature by simply ignoring them. Rather, we shall devote our attention to examining the individual theories of science and data that might be used to support or contradict them.

Several of the better known or more extensively used models of science are: "March of Science," "Revolutionary science," "Sociological view," and "Bumbling scientists" (see Intro).

In this essay, we have chosen to study a particular scientist, Hans Krebs, doing a particular piece of work, developing the "Citric Acid" or "Tricarboxylic Acid" or "Krebs" cycle. Ideally, what we can learn from this study can help to determine which, if any, of these models is satisfactory as an aid for understanding science.

In order to make this examination, we must decide where to go to obtain reliable data regarding scientific discoveries. Review of the work, or materials written by the scientist involved in the discovery are always suspect, since they are usually written well after the fact when the author's memory has faded, his conception of his own work modified by intervening events and discoveries, and his ego expanded. Nor are the actual papers necessarily reliable for although their content may give a reasonably accurate version of the author's "best" thoughts at the time they were written, scientific papers usually deliberately construe the development of a scientific idea, often ascribing teleology that was in fact not formulated until after an experiment was run, and, almost as a rule, ascribing scientific development a hypothetico-deductive type structure.

The original research notebooks are perhaps the most reliable source of historical data, yet they are also the least available. In this paper, only the first two sources were available, and as a result a certain amount of speculation that is more or less unsubstantial had to be included. However, published sources provided a wealth of information which, upon interpretation, has revealed much about how Krebs formulated and defended his theories on the oxidative pathway for pyruvate degradation.

The citric acid cycle, or tricarboxylic acid (TCA) or Krebs cycle, is the biological pathway that is responsible for the oxidation of pyruvate, the final product from the glycolysis of sugars, and is shown in Figure 1. It was proposed by Hans Krebs and William Johnson in a 1937 paper in *Enzymologia*[32] on the basis of their experiments performed on pigeon breast muscle tissue and work done by C. Martius and F. Knoop in 1936 (breakdown of citrate),[55-57] by Albert Szent-Gyorgyi between 1934 and 1936 (reduction of oxaloacetate to succinate),[61,62] Battelli and Stern[1] and Hans Einbeck between 1911 and 1913 (conversion of succinate to malate via fumarate), and Torsten Thunberg between 1917 and 1920 (the first sequence for the oxidation of acetate via the fusion of two molecules of acetate to form succinate followed by conversion via fumarate and malate to oxaloacetate and pyruvate to acetate).[64] Krebs' and Johnson's *Enzymologia* paper contained new experimental evidence demonstrating that citrate was generated in muscle tissue slices (as a result of malonate blocking of the succinate to fumarate step and that citrate catalyzed the absorbtion of 0_2 by the tissue). This paper was supported by a paper published later in the year demonstrating a similar citrate forming process *in vivo*,[34] and finally in a third paper published in February of 1940, which showed that fumarate catalyzed the oxidation of pyruvate and that the catalysis was inhibited by malonate.[37]

Sir Hans Adolf Krebs was born at Hildesheim, Germany in 1900, the son of an ear, nose and throat surgeon. In the usual European fashion, he received his university training at a number of institutions including Gottingen, Freiburg, Munich, and Berlin. In 1925 he received his M.D. degree at Hamburg, and then spent one year studying chemistry in Berlin. In 1926 he became an Assistant to Otto Warburg at the Kaiser Wilhelm Institute for Biol-

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ogy at Berlin Dahlem, where he worked for five years. Krebs said that he learned more from Warburg "than from any other single teacher."[49]

In 1932, Krebs became Private Dozent of Internal Medicine in Professor Thannhauser's Clinic at Freiburg where he worked out the biochemical pathway for the synthesis of urea. When the Nazi government came to power and dismissed Krebs in June 1934, Krebs was invited by Sir Frederick Gowland Hopkins to Cambridge, where he was supported as a Rockefeller Research Fellow and later as a University Demonstrator. Krebs continued to be supported by the Rockefeller Foundation for much of his scientific career. At Cambridge, Krebs continued work in amino acid metabolism. In 1935, Krebs became a lecturer in Pharmacology at the University of Sheffield and in 1938 a Lecturer-in-Charge of the Department of Biochemistry. It was at Sheffield that Krebs formulated the citric acid cycle in 1937, was married in 1938, and appointed Professor in 1945. In 1947, he was elected a Fellow of the Royal Society and in 1953 won the Nobel prize in physiology or medicine. In 1954 Krebs became the Whitley Professor of Biochemistry at Oxford.

In addition to ornithine and citric acid cycles, Krebs made numerous contributions to the understanding of biological pathways, remaining active even after his official retirement in 1967.

However, if we are to understand how the citric acid cycle was conceived, it is necessary to convert the biography from merely a collection of dates and places into something that will provide more information about the inputs into the theory. Presumably a variety of experiences that Krebs was exposed to influenced him in both his choice of scientific problem and his method of approach, and these specific experiences need to be examined as well.

It is relatively easy to identify the source of Krebs' experimental techniques. The manometric technique used to determine oxygen uptake (one technique in the 1937 *Enzymologia* paper[32]) came directly from Warburg's laboratory, as did the technique of using tissue slices. Krebs credits Szent-Gyorgyi for the idea of using pigeon breast muscle for the study of respiration.[51] Most of Krebs' analytical techniques were obtained directly from the literature, although he developed some new ones as well.

In Freiburg, Krebs collaborated with Kurt Henseit to put many of these techniques to use.[22] Krebs suggested that the successful formulation of the ornithine cycle in 1932 as a result of this work was a major influence in his formulation of the citric acid cycle. Certainly the "paradigms" established by this work were fundamental parts of the citric acid cycle, particularly that an appropriate way to establish the intermediacy of a compound in a

Fig. 1. Hans Krebs. (Wide World).

pathway is to demonstrate that it reacts in the same manner at least as fast as its precursor, and also the role of cycles in biological (and particularly catalytic) processes. Krebs in 1947 underscored this last point,[47] pointing out that based on the experience with the citric acid and ornithine cycles, a search for cycles is an appropriate "working hypothesis." (Whether in fact this hypothesis was operant in 1937 will be discussed more extensively later.)

It is more difficult to establish when Krebs first became interested in the problem of the oxidative breakdown of carbohydrates. In 1927 at the Kaiser Wilhelm Institute, Krebs published a paper on the "Role of Heavy Metals on the Auto-oxidation of sugar solutions,"[21] but this paper was cast in the more traditional style of arguing the existence of biochemical pathways based on analogies with organic chemical reactions, and it certainly was not the beginning of an effort that eventually led to the citric acid cycle. Krebs himself suggested in 1970[51] that he first carried out experiments in 1932 in Freiburg "on the oxidizability of substances which might be expected to be intermediates in the sugar breakdown pathway. Although he conceded that between 1933 and 1935 his "main research was on other topics—the oxidation of amino acids, the properties of D-amino acid oxidase, and the biosynthesis of glutamine," he suggested that the "problem of the intermediary stages of respiration remained in the forefront" of his mind as "one of the big unsolved problems of biochemistry." Nevertheless Krebs claimed that the 1932 work had left a "striking impression in my mind...that citrate, succinate, fumarate, malate and acetate were all readily oxidizable in various tissues." He also mentioned that his own efforts "in solving this problem (never published) were based on the idea that acetone dicarboxylate and acetoacetate might be intermediates," yet in 1935 when Szent-Gyorgyi began to publish papers on respiration in pigeon breast muscle, Krebs noted that Szent-Gyorgyi "failed to offer an adequate explanation of the effect of malonate which was known to inhibit cell respiration and to be a specific inhibitor of succinic dehydrogenase. This meant that succinic dehydrogenase must play a key role in respiration..." Finally, Krebs claimed to have observed the catalytic stimulation of respiration by citrate "just before" he read the paper by Martius and Knoop which outlined the formation of α-ketoglutarate from citrate.

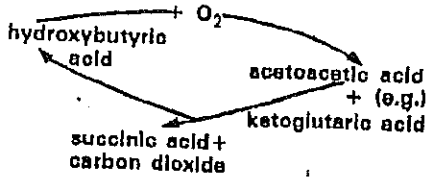
Although it is true that Krebs devoted much of his research time to amino acid metabolism,[23,24] it is likely that his conception of the carbohydrate oxidation problem was not as Kuhnian as his 1970 remarks would seem to indicate, nor were his ideas on the intermediacy of acetoacetate "never published." In fact, they appeared in a series of five papers submitted between July 9, 1936 and July 24, 1937,[26-31] the first of which was titled "Intermediate Metabolism of Carbohydrates."[26] Krebs clearly was concerned with the synthesis of succinate from pyruvate via α-ketoglutarate, and proposed the following

scheme as a mechanism for this synthesis:

1) CH_3COOH + pyruvate + ketonic acid ⟶ ketoglutarate + hydroxyacid

2) ketoglutarate + ketonic acid + H_2O ⟶ succinate + CO_2 + hydroxyacid

Krebs proposed that acteoacetate was the preferred ketonic acid inireaction 2, and proposed the following (cyclical) scheme to explain the formation of succinate from pyruvate:



In March of 1937,[27] Krebs adduced evidence for a reaction of type 2 in tissue slices, suggesting that reactions one and two "play a role in the normal oxidative breakdown of carbohydrates." In this paper Krebs made his first published distinction between oxidative (aerobic) and anaerobic pathways for disposing of pyruvate, a distinction that was to be of importance in the citric acid cycle.

In a paper submitted twenty-six days later,[28] Krebs' proposal had changed. Although he still argued that pyruvate could be converted into hydroxybutyrate, he proposed instead as intermediate the Claisen condensation product of acetate and pyruvate, acetopyruvic acid, which could then be converted into either acetoacetate or hydroxybutyrate. The evidence for this proposal was simply the ability of tissues to degrade acetopyruvate, further evidence of Krebs' faith in Hopkins' statement of 1911 that "the body is in general able to deal only with what is customary to it." In an effort to eliminate possible non-enzymic reactions of acetopyruvate, a number of organic chemical studies were done with the compound. In this new proposal, the concept of a "cycle" had been dispensed with.

In July of 1937[30] Krebs dealt specifically with the Szent-Gyorgyi proposal that fumarate, oxalacetate and pyruvate act as catalysts in cellular respiration, and worked with bacteria instead of animal tissues. Coming only five days before the title date of the *Enzymologia* volume that contained the citric acid cycle paper, and in view of the fact that Krebs always had referred to Szent-Gyorgyi's ideas as important to his development of the citric acid cycle, this is an important paper to examine to determine precisely what Krebs was thinking in 1937.

Krebs sets out to elucidate the following points:

1. Which substances act as hydrogen carriers in the cell?
2. The rate of hydrogen transport by a given carrier.
3. The nature of the chemical grouping donating hydrogen to the carrier.
4. The nature of the chemical grouping accepting hydrogen from the carrier.
5. The significance of a given carrier in cellular respiration.

The techniques used were no different from those used in previous and subsequent papers by Krebs. Eighteen conclusions were presented in summary, including the following:

"1. Fumarate, pyruvate, oxaloacetate, and probably carbon dioxide act as respiratory catalysts (hydrogen carriers)."

"5. Fumarate oxidizes anaerobically glucose, malate, lactate, acetate, glycerol, glyceraldehyde, pyruvate, butyrate, acetoacetate, glutamate and molecular hydrogen."

"14. Fumarate promotes catalytically the fermentation of pyruvate."[30]

Krebs echoed these conclusions in a paper published in 1937 in a book dedi-

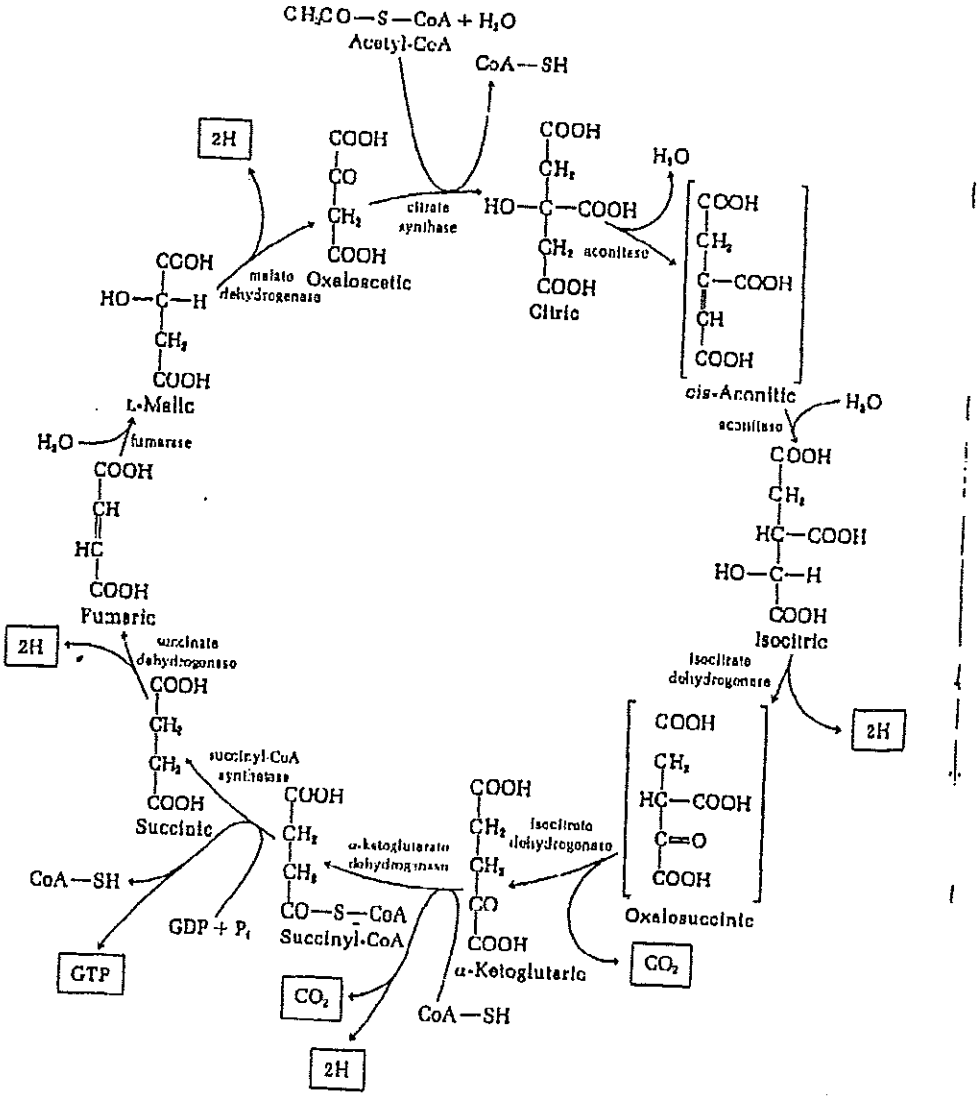


Fig. 2. The tricarboxylic acid cycle, as it is presently understood.

cated to F.G. Hopkins, where he wrote:

> "The work which is reported developed along lines similar to those which Szent-Gyorgyi discussed in his stimulating papers... . Oxaloacetic acid was the metabolite considered as the most important hydrogen carrier in the respiration of muscle tissue...We find it also acting in *Bacterium Coli*."

There are several things notable about these papers. First, they contain a wealth of data that could be used to establish a citric acid cycle, lacking only the compounds citrate and α-ketoglutarate. Also, from an overview of these and the papers preceding them, it seems that Krebs had initially attempted to impose a cyclic structure for the mechanism of carbohydrate oxidation by either conscious or unconscious analogy with the ornithine cycle, but had failed. More important from a philosophical point of view, the papers were merely extensions of work by Szent-Gyorgyi, and not very significant ones either. There is no sign in either paper that Krebs found the proposal of Szent-Gyorgyi in any way problematical, nor is there any sign that Krebs felt the mechanism for the oxidation of pyruvate to be "one of the big unsolved problems of biochemistry." Also contrary to Krebs' 1970 conception of the steps leading to his discovery of the citric acid cycle, there is no evidence that his 1932 experiences had left in his mind a "striking impression that citrate...[was] very readily oxidized in various tissues."[61] From a Kuhnian point of view, Krebs in 1937 was as "normal" as a scientist can get, and it also seems clear that Krebs' 1970 recollections are not of high historical fidelity.

Between the July 24 paper and the July 29 issue of *Enzymologia* there is a hiatus of at least five days, and the only primary source of information available for this period comes from the laboratory notebooks of Krebs and Johnson. During this gap, Krebs had an opportunity to read a March 1937 paper by Martius and Knoop[56] which described the metabolic fate of citrate in biological materials and specifically the fact that α-ketoglutarate was an oxidation product of citrate. Martius and Knoop were also "normal" scientists; they were studying a normal science problem with normal science techniques, and Krebs had never had occasion to reference any of their previous work. Yet it is easy to see how this paper tied in with all of Krebs' work of the previous five years, and it is possible to speculate on what thoughts the Martius and Knoop paper stimulated in Krebs' mind.

First, "α-ketoglutarate" was a magic word, having been proposed by Krebs as a crucial intermediate in the 1936 cycle published in *Nature* on the

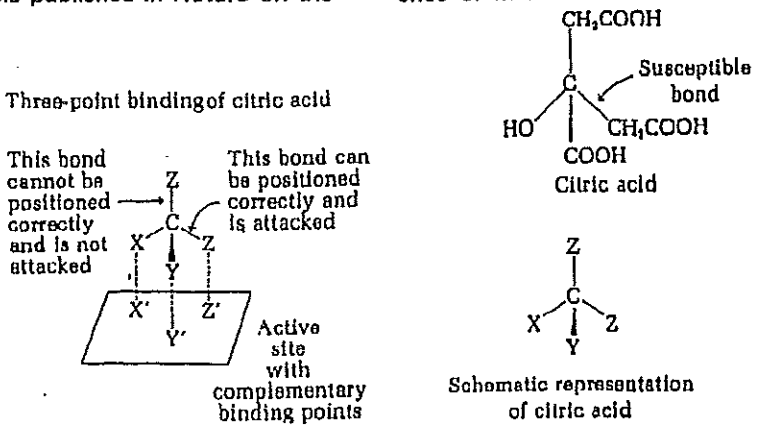


Fig. 3. The asymmetric action of aconitase on citric acid, as explained by A.G. Ogston in 1948. His explanation led directly to the final acceptance of Krebs' original formulation of the TCA cycle.

"intermediate metabolism of Carbohydrates,"[26] "Cycle" was also a magic word, ever since the ornithine cycle, and Krebs had abandoned his idea of a cycle for sugar oxidation only five months earlier. At that time (in the March 1st and 27th papers) Krebs had been looking for an appropriate substance to condense with pyruvic acid to end up at α-ketoglutarate. Of course, the substance that pyruvate had to condense with to form citrate was none other than oxaloacetate, the very compound that just a few weeks earlier Krebs had concluded was "the most important hydrogen carrier in the respiration of muscle tissues."[31]

"Catalyst" too was a magic word, since the ornithine cycle had been established by the ability of citrulline to catalyze the formation of urea, and in July Krebs probably realized for the first time that neither Szent-Gyorgyi nor Stare and Baumann could explain the catalytic effect of fumarate on respiration. With the cycle thus established from fumarate to succinate, it only required a distinction between the reductive conversion of fumarate to succinate (which Szent-Gyorgyi had elaborated on barely six months before) and the oxidative conversion of succinate to fumarate, a distinction that Krebs had made twice in the previous year, before the complete cycle could be established and the catalytic effect explained. Finally, it was probably at this time that the significance of malonate inhibition was realized, after the initial experiments Krebs ran showed fumarate converted almost quantitatively to succinate in the presence of malonate.

It should be noted that the citric acid cycle thus formulated was merely a list of compounds, each of which has a history of its own. Some of the intermediates, including fumarate, malate, succinate, and citrate had long been known to organic chemists.[14] Others, including cis-aconitate, isocitrate, and oxalosuccinate were much more recent additions to the organic chemist's repertoire, yet none of these compounds was important to Krebs in 1937. This is readily apparent from the fact that in his December 1937 summary of the citric acid cycle,[34] Krebs mentions only succinate, fumarate, malate, oxaloacetate, citrate, and α-ketoglutarate. The only compound that was relatively new in this scheme was also quite likely the compound that triggered the citric acid cycle idea, α-ketoglutarate, and for these reasons it is interesting to digress a moment to trace its history.

The systematic knowledge of α-ketoglutarate (AKG) goes back only until 1908, when its synthesis was reported by two Frenchmen, F.E. Blaise and H. Gault.[3] Their motiva-

tions for synthesis were simple and mundane.

> The Ketoacids, although known for the malonate and succinate series, are not known for diacids containing more carbon atoms, except for the pimelic acid series. We have therefore shown that the α, α-diketo pimelic can be obtained by saponification of alcoylidene-bisoxalacetic esters in mineral acid medium. We decided to try to generalize this work to also prepare the α-monoketo and α, α-diketo diacids that have until now been unknown.[3]

The authors thought their work publishable because it embodied a new synthetic technique. They have found that the method of Wislicenus[66,67] for synthesizing alpha keto acids by saponification, a procedure published twenty years earlier, did not work for the compounds they wished to prepare. Blaise and Gault achieved the hydrolysis with cold acid instead of base.

The interest in alpha ketoglutaric acid in subsequent years was primarily of a chemical nature. *Beilstein* lists some twenty references between 1910 and 1930, including papers by Haworth and Gabriel[15,19,20] in which AKG was used as a starting material for several organic syntheses. In 1915, Carl Neuberg and M. Ringer at the Kaiser Wilhelm Institute determined that yeast could ferment AKG;[59] this was the first of a number of papers that examined the role of AKG in biological systems. However, like citrulline and cis-aconitate,[54] AKG was a chemical studied by chemists before it became a biochemical examined for its role in biological systems.

Despite its formal similarity to the ornithine cycle, it should be noted that the citric acid contribution was quite distinctive in nature. In the case of urea synthesis, Krebs' series of experiments carefully elucidated a pathway that was hitherto unknown. The citric acid cycle was more of a conceptual breakthrough in which Krebs organized a variety of previously known but incoherent facts, providing experimentally only the evidence for the formation of citrate from pyruvate and oxaloacetate. Thus, although many, including Krebs himself, have since noted the similarity between the two cycles, from the point of view of their development (and, as we shall see in the next section, their acceptance by the scientific community), they were very different.

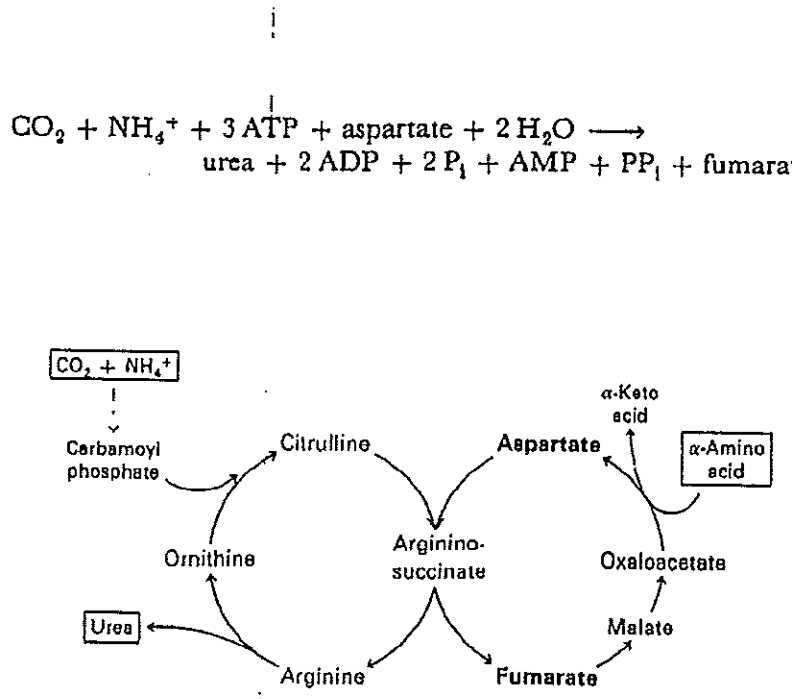


Fig. 4. The metabolic pathway for the synthesis of urea. The cyclic nature of urea production may have influenced Krebs when he postulated a cycle for the intermediary metabolism of carbohydrate oxidation.

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The original Krebs and Johnson paper, as discussed above, assigned a crucial role to citric acid as an intermediate in carbon metabolism, both in the title and in the body of the paper. Krebs named his cycle the "citric acid cycle," provided the reader with no fewer than four diagrams showing citric acid as an intermediate, concluded that "the quantitative data suggest that the 'citric acid cycle' is the preferential pathway through which carbohydrate is oxidized in animal tissues," and pleaded ignorance about whether cis-aconitate is involved in the pathway. There was only a single scheme for carbohydrate metabolism considered or suggested.

Immediately following the publication of his *Enzymologia* paper, Krebs published a series of papers with various co-workers in which he adduced further evidence in favor of his cycle.[34-36] The first such follow-up, received by the publisher in December of 1937, contained a diagram showing the citric acid cycle to contain only citrate, α-ketoglutarate, succinate, fumarate, malate, and oxaloacetate. The purpose of the paper was to "supplement the previous evidence" derived from isolated tissues with evidence from "experiments on the intact organism." As such, the work was straightforward and Krebs' comments were not unusual, except for a brief postscript added January 8, 1938. In this postscript, Krebs noted that:

> Breusch has recently questioned

the existence of the "citric acid cycle" in muscle tissue. We are, however, unable to accept his arguments and shall discuss them in full in a following paper.[34]

F.L. Breusch was at the University of Szeged in Hungary (also the home of Szent-Gyorgyi) when his first paper attacking the cycle was published.[4] Like Krebs, Breusch was fleeing National Socialism and the impending war; in 1938 he moved from Hungary to the Department of Physiology at the University of Istanbul. There he published a second paper on the citric acid cycle,[5] joined in his attack by Jacques Thomas,[63] who was then working in the laboratory of E.J. Bigwood at the University of Brussels. The basis for their dispute was simple; both workers felt that the citrate that Krebs had detected had been "artificially produced under the conditions of their experiments." Thomas wrote:

> Muscle has, in confirmation of my previous work and contrary to the citric acid cycle of Krebs, no ability to form citric acid. It is futher shown, in confirmation of muscle perfusion experiments of Martensson, that muscle has only slight ability to break down citric acid, again contrary to the cycle theory.[63]

Both experimentors had added precursors of citric acid to various tissue and attempted to detect an increase in the amount of citrate in the tissue. Neither had found the yield of citrate ever to exceed 2%. Thomas had noted that the catalytic effect of citrate on oxidation occured only after an hour wait, whereas that exerted by fumarate was instantaneous. Finally, Breusch had argued that citric acid could not be an intermediate in any cycle, since muscle could not degrade citrate sufficiently quickly, finding that less than 16% of the citrate added to muscle disappeared, and that only "due to a balance between citric and isocitric acids."[5]

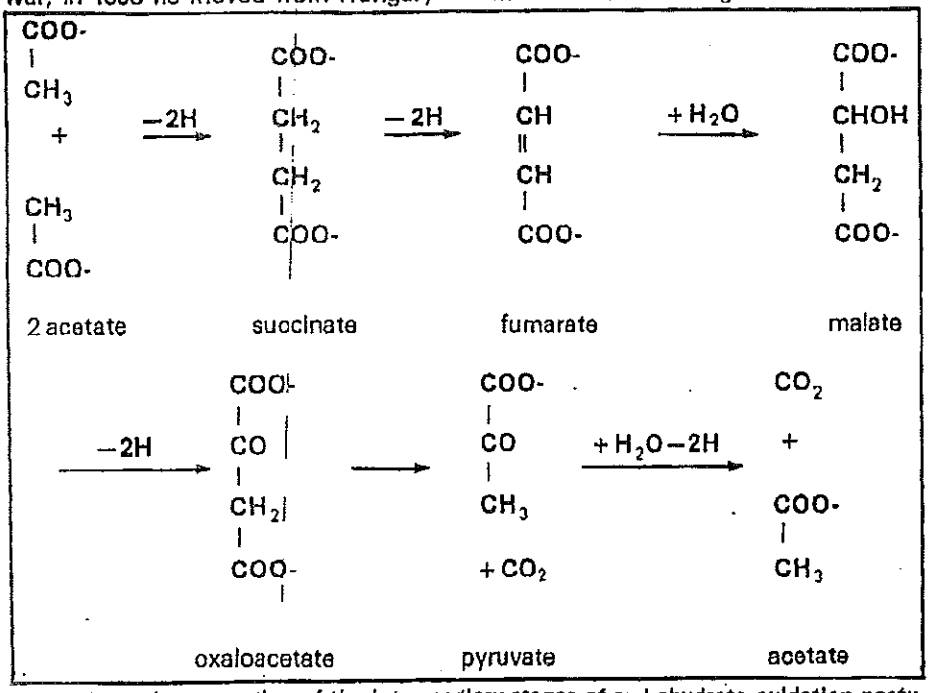

Fig. 5. An early conception of the intermediary stages of carbohydrate oxidation postulated by Thunberg and supported by Knoop and Wieland in the early 1930's.

Krebs' "subsequent paper" took over two years to appear, but it was a concise and conclusive refutation of his critics.[37,38] Accusing "both authors" of disregarding "one of the most important experimental observations in the field, viz. the oxidative formation of succinate from oxaloacetate in the presence of malonate," Krebs for the first time challenged seriously the theory that underlay Breusch's and Thomas's conception of biochemical events, that of Szent-Gyorgyi. As for the fact that high yields of citrate could not be isolated from muscle to which oxaloacetate had been added, Krebs caustically pointed out the difference between the rate of accumulation of citrate and the rate of formation of citrate. With respect to Breusch's finding that muscle cannot break down citric acid, Krebs wrote:

> To refute Breusch's recent view, it will suffice to quote Breusch's own previous observation. He found that 20 ml of muscle suspension removed 20 mg of citrate...This rate of citrate disappearance can easily account for the total respiration of the muscle.[38]

Krebs polished off his criticism of Breusch and Thomas by noting that "several of the ciriticisms arise solely from misinterpretation of the theory," and that their experiments were "carried out on tissue which had already lost the greater part of its metabolic activity; thus it is not surprising that the results were unsatisfactory."[38]

In the background of all of these discussions was the principal concept that Krebs had to refute, that of Szent-Gyorgyi. As discussed earlier, Szent-Gyorgyi had postulated a hydrogen transfer system that included the sequence oxaloacetate, malate, fumarate, to succinate, a postulate that had in part won him a Nobel prize in 1937. At that time, the Nobel presentation speech delivered by E. Hammarstein, had noted that in Szent-Gyorgyi system "the flaws are numerous...but not of a character to breach the highway of the oxidation chain."[1]

As already mentioned, Krebs had for several years done work in support of the Szent-Gyorgyi pathway and was very slow in making the break complete. In both 1937 papers,[32,34] Krebs offered no criticism of the pathway. In February of 1940, he accepted Szent-Gyorgyi's theory only for "the conversion of triose into pyruvate,"[38] but nevertheless argued that the results of the experiment with malonate inhibition "calls unequivocally for a revision of this theory." Yet, in April of 1940 he incorporated the "Szent-Gyorgyi cycle" into a diagram of the citric acid cycle as part of the process that oxidizes citrate. It was only by 1943 that Krebs could write that the Szent-Gyorgyi theory "cannot be regarded as satisfactory" as a "theory of carbohydrate oxidation."

Krebs thus successfully refuted his

critics arguments by 1) relying on confirmable experimental data, 2) incorporating minor details into his pathway (for example, note how the addition of isocitrate clears up Breusch's contention that the citrate-isocitrate equilibrium is catalytically not important) and 3) distinguishing between the oxidative citric acid cycle and any reductive pathways that might employ components of the cycle. By the end of 1940, at least with respect to the principal aspects of the citric acid cycle, Krebs had triumphed.

That was not to be for long. In 1941, two groups of American scientists independently reported experiments with carbon isotopes in which a label was incorporated into the carboxyl carbon of oxaloacetate. By measuring the amount of label in the CO_2 released when α-ketoglutarate was decarboxylated, they determined that all of the label incorporated was released. This was contrary to the expectation that, because citrate was a symmetric molecule, only half of the label should become CO_2, and led directly to the conclusion that neither citric acid nor any other symmetric compound could be an intermediate in the formation of α-ketoglutarate from oxaloacetate.[10-12,68]

There is no doubt that removing the citric acid from the citric acid cycle was traumatic for Krebs, but it was the least extensive of a number of modifications proposed by the Americans. Wood et. al. suggested that the objection to a symmetric intermediate could be removed if oxaloacetate and pyruvate were condensed directly to yield cis-aconitate, with citrate being an unimportant side reaction.[68] However, Evans and Slotkin did not think even this probable in view of the equilibrium between citrate, aconitate, and isocitrate that overwhelmingly (90%) favors citrate.[11] Thus, they presumably were in favor of an indefinite although undoubtably more extensive revision of the original theory.

Not surprisingly, Krebs chose the first option. In 1941[43] he published a brief note titled "Modified Citric Acid Cycle" in which he accepted the hypothesis that "the formation of citrate is due to a side reaction." His response to the equilibrium objection was decidedly *ad hoc*. "[Evans and Slotkin's] argument only holds if the equilibrium

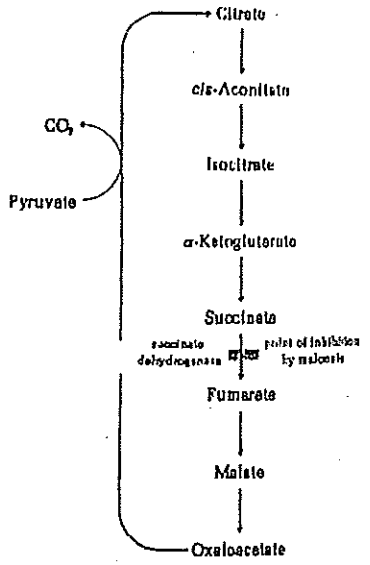

Fig. 8. Inhibition by malonate results in the accumulation of succinate. This suggested to Krebs that oxaloacetate reacts with pyruvate, producing citrate, a precursor of succinate.

is actually established."' Krebs then argued that it is in fact not established because of the relative slowness of the conversion between citrate and cis-aconitate. It is interesting to note that by arguing that this reaction is slow, he is essentially agreeing with Breusch and Thomas and disagreeing with the Krebs of 1940 who argued that the conversion of citrate to aconitate was rapid. Of course, the entire argument of Breusch and Thomas was that citrate was the product of an unimportant "side reaction." Although Thomas was not publishing at the time (World War II was in progress), this fact was not lost to Breusch in Turkey. In 1943 he wrote:

> According to Krebs' first opinion, all carbohydrate metabolism passes over the citric acid cycle. Later on he gave the cycle as an accounting of only 60 per cent of the metabolism, saying that for every cycle passed, the oxalacetic acid is reduced two times to l-malic acid. Krebs is now of the opinion that no citric acid is produced, but cis-aconitate formed by condensation of oxalacetic acid together with a sugar breakdown product.

Krebs was not willing to give Wood and co-workers full credit for the modified cycle; however, in 1943 he realized that he could claim priority for the modification as well. In his paper in *Advances in Enzymology*, he wrote that the Wood modification was in fact one of several options that he considered from the start:

> It was made clear [in 1937] that the [condensation product] chosen... was somewhat arbitrary. The arbitrary formulation was preferred because it was thought for reasons already stated, that a place for the reaction citrate ⇌ cis-aconitate should be found in normal muscle tissue (although this...does not imply that the reaction is part of carbohydrate oxidation).[44]

Not surprisingly, Krebs uses the title "citric acid cycle" in quotations, and calls the modified hypothesis the "Tricarboxylic acid cycle."

It is disputable whether in 1937 Krebs considered citrate as a side product as "one of several possibilities." In his paper in *Enzymologia*,[32] Krebs only questions "whether [aconitate] is an intermediate in the breakdown or synthesis of citric acid." In his paper in *Lancet*[33] of the same year, the conversion of oxaloacetate to citrate is shown to proceed via "oxalomesaconic acid" and "cis-aconitic acid," based on chemical models for the biological reaction. However, because of the name Krebs gives his cycle, the absence of any published writings to the contrary, and the extent to which Krebs defends the role of citrate in the cycle between 1937 and 1940, although it is clear that some consideration was given to the order in which citrate, aconitate, and isocitrate arise, it seems also clear that the Wood et. al. hypothesis is not a "minor modification" of the cycle "already contemplated from the state as one of several possibilities," contrary to Krebs' 1943 paper.

It might be said facetiously that for the five years following 1943, Krebs' Nobel prize hung in the balance. Krebs turned to studying other biosynthetic problems, primarily the fixation of carbon dioxide in heterotrophs and the metabolism of acetoacetate.[45,46] In papers on both topics, he heavily and respectfully referenced papers by

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Wood, Evans, and Breusch. But it was also during these years that other basic work was done on the cycle, work that would participate in its re-emergence in its original form. Following the war, Fritz Lipmann identified coenzyme A,[52] finally establishing its structure in 1952,[53] the year before he won a Nobel prize for the work. Much work with isotopes was also done to clarify intermediate steps in the reaction. Also, more traditional biochemical evidence continued to accumulate to supplement the 1940 paper by Krebs and Eggleston. Even Breusch contributed evidence of this type when, in 1946, he conducted a series of experiments and noted that "In all tissues investigated, the capacity for metabolizing citric acid is nearly parallel to the capacity for reducing oxaloacetic acid to malic acid."[7] Krebs himself published a paper on the "Microdetection of Isocitric and Cis-Aconitic Acids in Biological Materials."[46] He listed under "Applications" for this new technique the detection of isocitrate in plants; there was no mention of the "citric acid cycle," now the "TCA" cycle. All of the textbooks that this writer has seen that were written during this period listed citric acid as a minor side reaction.[16]

This state of affairs ended in 1948. In that year, A.G. Ogston of Oxford published a brief note in which he pointed out that through a "three attachment" scheme, an asymmetric enzyme could in fact distinguish between "identical" residues in a "symmetric" molecule. He concluded that "asymmetric occurance of isotope in a product cannot be taken as conclusive evidence against its arising from a symmetric precursor."[60] Although Ogston's paper filled fewer than eight inches of column space in *Nature* (including diagrams), it instantaneously and effectively ended the criticism of the citric acid cycle. It was as if the collective scientific community had gone "oops!"—and well it might have, for Ogston's paper could have been written by anyone in almost any field. It required no experimental work, and the concepts were merely extensions of the concepts of chirality first introduced by van't Hoff and Fischer and of the "polyaffinity" theory developed by Bergmann and co-workers. All it required was an author who could extend these concepts, draw together what were then diverse facts to bear on a single problem, and then formulate a new idea in what must have been an ideal example of scientific genius. Ogston did all of these things for the citric acid cycle, as he had done for many other scientific problems that he tackled.

If Ogston was the hero of the breakthrough in 1948, Krebs was its chief beneficiary. No one continued the attack on the citric acid cycle, or the TCA cycle, both now becoming better known as the "Krebs' cycle." The international acclaim came quickly, with Krebs giving a Harvey Lecture in 1950[48] and a Herter Lecture at the Johns Hopkins University in 1953.[49] Even Breusch in 1952 delivered a paper at the 2nd International Conference of Biochemistry in Paris, (titled the "Symposium sur le Cycle TCA"), in which he discussed his work on fat metabolism and his reaction to "recent concepts from the findings of the tricarboxylic acid cycle."[8] The principal award was the Nobel prize in Physiology or Medicine, awarded to Krebs in 1953 with Lipmann. The Presentation speech made by E. Hammarsten at Stockholm noted that "In the beginning Krebs was quite alone with his idea," yet also was historically simplistic by saying that Krebs' "penetrating intuition was so clear and his grasp of the problem so keen from the start that none of his original ideas had to be revised."[18]

The 1950 Harvey Lecture[48] was of particular interest because in spite of the fact that Ogston's three point attachment proposal had been available for over a year, it is not apparent that Krebs fully understood it. He began his discussion of the TCA cycle by arguing that "evidence bearing on the [order in which the tricarboxylic acids arise] became available in 1941 from isotope experiments by Wood..." even though the issue was not the sequence of acids but whether citrate was one intermediate in the pathway and despite the fact that Ogston had shown Wood's isotope experiments irrelevant to this subject anyway. Krebs then discussed at length Wood's analysis, concluding that he suggested "that a minor modification, already contemplated from the start as one of several possibilities, would meet the facts," despite the fact that 1) the modification that Wood suggested had not been considered from the start (the hypothesis that citrate was a side-product), and 2) that Ogston had shown that the modification was not needed to meet the facts. Krebs then proceeded to note that "the main premise upon which this modification rests is the relative slowness of the formation of citrate from cis-aconitate. This premise has a sound experimental basis." Krebs then proceded to establish Wood's modification as if he had either not read or not understood Ogston's proposal.[48]

It is only after establishing citrate as a side product that Krebs, in contradiction with the prevous two pages of his essay, mentioned that Ogston had shown that "citric acid does not necessarily behave as an asymmetric (sic) molecule when combined with an enzyme." Krebs then quotes verbatim some passages from Ogston before finally explaining in his own words what "three point attachment" meant to him, concluding finally that "the conclusion that citric acid cannot be an intermediate cannot be accepted as binding."[48]

Nevertheless, by 1950 Krebs was sufficiently confident of the validity of the TCA cycle to assert that "the original scheme received some elaborations, but in all essential aspects the original scheme still stands." By 1953, in his Nobel address, Krebs thought the controversy of ten years before unimportant enough to ignore it altogether.[49] By 1970, Krebs summed up the struggle for the acceptance of the citric acid cycle by saying that "like almost any new concept, the idea of a TCA cycle was severely criticized by some biochemists, but the majority soon accepted it as a working hypothesis."[51]

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The most striking fact about the history of the Krebs cycle is the unreliability of sources written after the fact to tell accurately how a scientific idea was formed and accepted. Examples of this unreliability range from the trivial, that Krebs original ideas concerning the metabolism of carbohydrates were not published when in fact five articles

were published on the subject, to the much more complex, when precisely Krebs performed experiments testing the catalytic activity of citrate in pigeon liver. The second point needs some elaboration, since I have implicity questioned Krebs 1970 contention that he had performed the crucial experiment "just before" reading the paper of Martius and Knoop. The questioning was implicit only because the laboratory notebooks which would be the only reliable source of an answer are unavailable. It seems reasonable to say that Krebs tried the citrate experiments as a result of Martius and Knoops' paper, but if this were not the case, it is difficult to explain why Krebs did test citric acid for catalytic capabilities. He could not have tested it in an attempt to find a condensation product of pyruvate and "something else" (the reason he tested acetopyruvic acid in March of 1937), because he would have had to realize that the "something else" was oxaloacetate, and would have come across the cycle without the help of Martius. In fact, it seems that any but the most "let's try it and see" attitude by Krebs towards citrate would have crystallized the citric acid cycle concept immediately.

The retrospective view of Krebs and others concerning the acceptance of the citric acid cycle contain even greater historical blind spots that would mislead one who did not refer to primary sources. In all of his published writings, Krebs in 1943 was persistent in insisting that the modification that Wood had proposed for the cycle had been an alternative he had considered all along.[44] He managed to do this by confusing an alternative order of the tricarboxylic acids which he in fact might have considered, with Wood's proposal that citrate was not an intermediate in the pathway, something he clearly did not consider even when Breusch and Thomas argued so forcefully for that proposal. Whether Krebs' obfuscation in 1943 was deliberate can not be said for certain, but it continued even until the 1950 Harvey Lecture, when he discussed the isotope labeling experiments under the heading "Sequence in which the tricarboxylic acids arise."

Krebs was not the only one who forgot the details of the citrate controversy, as was shown by the 1953 Nobel prize introduction that read that Krebs "penetrating intuition was so clear and his grasp of the problem so keen from the start that none of his original ideas had to be revised." It is clear that a reflective "history" of a scientific discovery should be regarded only as another historical document, and not as an authoritative factual source.

The historical discussion also provides us with some data which can be used to examine the models of scientific discovery that were mentioned in the *Scientific's* introduction. First, despite the statement of Professor Hammarsten to the contrary, intuition seems to have had little to do with the formulation of the citric acid cycle nor was chance particularly important. Krebs in 1937 certainly had a "prepared mind," and he is probably too modest when in 1970 he explains the contrast between his success and Martius and Knoop's failure at putting together the cycle as being merely a result of the fact that he was a biochemist while Martius was a "theoretical organic chemist."[51] Both in terms of chemistry and biochemistry, Krebs clearly was familiar with a broad spectrum of the then current scientific literature.

The concept that Krebs was participating in a broad "march of science" can only be justified as a way of looking at history with the benefit of hindsight, and Krebs' characterization of the discovery of the citric acid cycle as a "slow evolutionary process extending over five years" must be viewed in this way. The Kuhnian "revolutionary science" model should be discussed more extensively. It is not difficult to argue that the discovery of the citric acid cycle was a scientific revolution. It is still placed at the center of the metabolic pathway chart simply because so many of the elements of the cycle are at the end or beginning of other important synthetic pathways. It provided the impetus for numerous other discoveries including that of Coenzyme A by Fritz Lipmann. It provided the organization for a new conception of biosynthetic pathways, and established looking at biological systems as a way of demonstrating pathways, not merely looking at analogous organic reactions.

However, if all that the Kuhnian model postulated was that some research papers are more important than others, it would be trivial; as a theory of scientific advance, it argues for much more. Kuhn first makes a distinction between "normal" science and "revolutionary" science. Normal science operates under a paradigm, while revolutionary science occurs in a crisis situation when the paradigm is challenged in the face of conflicting data. As has already been discussed, there was no crisis in paradigm or in anything else in 1937; the elucidation of the mechanism of carbohydrate oxidation was viewed as a problem for normal science. If the papers of Krebs up until July of 1937 are any indication of his thoughts at that time, Krebs saw no evidence that conflicted seriously with the theory of Szent-Gyorgyi. There was a scientific problem, but no more of a problem than any other that Krebs had examined.

Even more startling by its absence is the "Kuhnian" revolution represented by Ogston's paper. Once again, in scope Ogston's paper was clearly revolutionary; it initiated the first examination of prochirality in enzyme systems, an examination that culminated in the elegant work of Westheimer just a decade later. Conceptually as well it was an abrupt change with the past. However, there was absolutely nothing that could be regarded as a "crisis" prior to the revolution. It is true that the Wood and Evans proposal had created a problem in the TCA cycle, but it was a problem for normal science and not a very serious one. The only research work being done in this particular aspect of the cycle were a few isolated attempts to determine the mechanism by which "pyruvate" condensed with oxaloaceate. Thus it seems clear that neither revolution can be called Kuhnian.

The socio-economic model of scientific discovery also cannot be discarded easily. Certainly Krebs was interested in establishing himself in England after what must have been a very upsetting experience with considerable hardship, and it might also be relevant to point out that Krebs was not married at the time of his discoveries, both socio-economic factors that affect commitment in the laboratory. However, the support of the Rockefel-

ler foundation gave Krebs a degree of financial security, and there is no evidence that there were any factors that greatly influenced Krebs' performance other than an intellectual ambition and commitment to diligent work that are generally characteristic of any good scientist. In 1970 Krebs commented on this subject, saying that "the sense of competitive 'racing' was unimportant" to him and since he was using new "homemade" research tools, he felt that "it was most unlikely that anybody else at that time would make the same approach." Krebs then lists three basic motivations—an "insatiable curiosity," an "ambition to justify my choice of career as a scientist vis-a-vis those who were doubtful about my ability to make a success in this field," including his father and Warburg, and a desire to satisfy those who supported him financially.[51]

The "bumbling scientist" model (from Webster, "bumbling: self-important in a blundering sort of way") is this author's preferred model of scientific advance. The development of this model was aided by a conversation I had with Dr. Fruton, during which he suggested that the proper way to regard scientists is not with awe or disdain, but rather with sympathy, since in most cases they are tackling problems that are too hard with concepts that are too feeble and tools that are too primitive. In other words, science is too hard for scientists.

Unfortunately, this model is the most temporally provincial of all. By postulating that the scientists of 1937 did not know what they were doing, it implies that someone else knew better, an implication which is clearly false. Futhermore, it is evident that scientific advances are made, so a scientist cannot be "bumbling" all of the time. In fact, even with the benefit of hindsight, the papers of Krebs and Ogston are in virtually every way excellent examples of quality experimentation and thinking. Thus, while scientists are not infallible, they constantly display a degree of competence that few other professions can match.

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