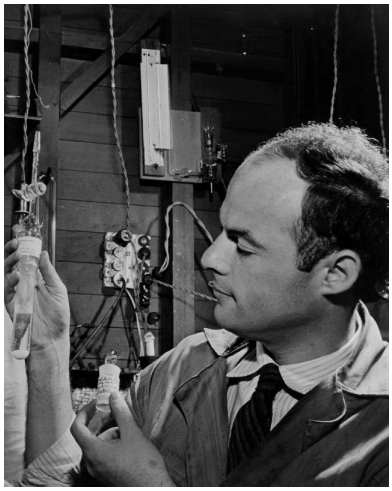
A Vicious Cycle: Melvin Calvin, Andrew Benson, and the Path of Carbon in Photosynthesis

Discussion questions are in boxes. Suggested answers are in *italics*. These are meant to help guide discussion, not to serve as a key for the evaluation of student responses as "right" or "wrong."

Melvin Calvin and Andrew Benson

The discovery of the nature of radioactivity and the identification of the neutron sent shock waves through the discipline of chemistry. Traditional atomic theory held that all atoms of a given element are identical, all carbon indistinguishable. This gave little hope to any scientist who wished to trace the path of individual atoms through a system as complex as a living cell. In 1946, Melvin Calvin was tasked with doing exactly this. He was hired to work at the Berkeley Radiation Laboratory by Ernest O. Lawrence, whose name the laboratory now bears. Calvin was put in charge of a research group tasked with determining the path of carbon in photosynthesis; to this end, they would use radioactive carbon-14 as a tracer. The use of radioisotope tracers was a recently developed technology, made possible by cyclotrons (early particle accelerators), of which Berkeley had one of the first. Andrew Benson said that carbon-14 had been "invented," not discovered, by Ruben and Kamen, since its application as a research tool predated its observation in the natural world. They "invented" it via the ingenious and comparatively low-tech means of setting flasks of ammonium nitrate next to the cyclotron, so that the nitrogen atoms would soak up stray neutrons produced in the normal course of the machine's operation. Virtually the entire world's supply of carbon-14 had been left to Calvin as a grim inheritance following Sam Ruben's tragic death in a laboratory accident involving a wartime chemical weapons research project. The Calvin group's use of this new tool to elucidate a fundamental biochemical pathway was an unprecedented intersection between disciplines in biology, chemistry, and physics.



Sam Ruben, holding the world's supply of manmade carbon-14

Calvin got his start in chemistry with an undergraduate degree from Michigan College of Mining and Technology (now Michigan Technological University); because the chemistry program was relatively new and its curriculum limited, Calvin "was not encumbered with preconceived notions of how the actual biochemistry of living systems worked," as he would later state in his autobiography. (Ironically, history's 20/20 hindsight would later reveal that Calvin was encumbered with many preconceived notions about how chemistry worked in the abstract—preconceptions that would alternately help and hinder his discoveries.) Calvin's postdoctoral work at the University of Manchester under Michael Polanyi involved redox reactions of porphyrins, biological molecules with coordinated metal ions in their centers (like chlorophyll and hemoglobin). Polanyi is best known today for being the first to develop the theory of transition states in reactions, and Calvin's work with him would help establish his credibility as an authority on this as well.

In 1937, Calvin was hired appointed assistant professor at UC Berkeley by Gilbert N. Lewis, the legendary, cigar-chomping chair of the chemistry department (best remembered today for developing the Lewis structure). Lewis was convinced to hire Calvin by Joel Hildebrand, another UC Berkeley chemistry professor who had visited Manchester and become acquainted with Calvin's work there. At the time, Lewis was researching the absorption and reemission of light by organic molecules, and Calvin's background was a natural fit. The two collaborated on several investigations to this end.

In the years prior to Calvin joining the Rad Lab group, Ruben and Kamen were doing groundbreaking work on radioisotope tracing. It was known that photosynthesis consisted of two stages, the “light reactions,” which required light and water and generated oxygen, and the “dark reactions,” which consumed carbon dioxide, and could proceed (at least for a short time) in the absence of light. Little was known about the connection between the two, or what exactly was created by the light reactions that the dark reactions required.

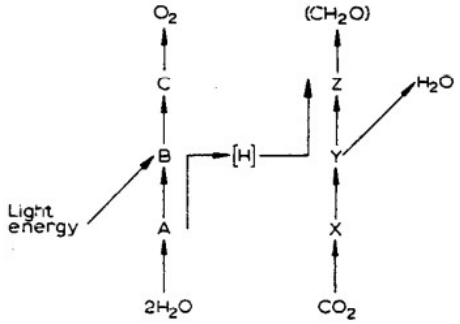


Fig.1. Elementary photosynthesis scheme.

Martin Kamen had access to Berkeley's 37-inch cyclotron, but was relegated to using it only after the university's physicists had left for the day. Ruben and Kamen's early experiments used ^{11}C, which has a half-life of only 20 minutes. (This necessitated a literal sprint to the laboratory after each run of the cyclotron.) Using ^{11}C tracers, Ruben and Kamen had determined that the first step of the dark reactions involved the fixation of CO_2 into an initial product with a carboxyl group.

Andrew Benson, a biochemist, briefly joined them in 1942. Soon afterwards, though, Benson's position at Berkeley became untenable—he was a conscientious objector, and the chemistry department was involved in the Manhattan Project and chemical weapons research. Benson worked for Forest Service for a time before moving to Stanford to work on antimalarial drugs; later, he moved to Caltech. Caltech held special appeal for Benson; he'd earned his Ph.D. there, and his wife's family lived in Pasadena. While there, he met Sam Wildman, which gave rise to an ongoing collaboration.

Benson returned to Berkeley in 1946, when Calvin hired him as associate director. 1946 was a mixed bag for the group. The Manhattan project had concluded, making cyclotrons and isotopes available for civilian applications again; obtaining carbon-14 was no longer an issue. The war, too, was over, but not without incurring casualties in the chemistry department. Ruben was killed handling phosgene during a chemical weapons experiment, and Kamen lost his job when he (groundlessly) fell under suspicion of associating with Russian spies.

Let's consider a hypothetical pathway involving a series of reactions, x → A → B → C → D → y etc. Suppose x represents the light reactions and A through C represent the steps between carbon fixation and glucose synthesis.

- Of the above intermediates (x, y, and A-D), which would you expect to be present in a normally functioning plant cell? Why? (*All of them; a plant performing photosynthesis would have some molecules present at each step of the process.)*
- If you want to study the progress of the steps, you'll need initial conditions where concentration of each is set at (or near) zero. How could you accomplish this in a living plant? (i.e., how could you prevent carbon fixation?) How will this need inform the design of the container the plant is kept in? *(By denying the plant CO_2. This could be done in a sealed flask. CO_2-scavenging reagents could be added, or the air in the flask could be flushed out with an inert gas, etc. The flask would need to be airtight, but also have a valve to allow the introduction and removal of gases.)*
- If you prevent carbon fixation, how will this affect the concentration of the products of the light reactions? *(They will accumulate.)*
- Once carbon fixation is allowed to continue, would you want the plant to be kept in the light or in the dark? Why? *(In the dark. If the plant was kept in the light, then Substance A would be continually replenished, because the products of the light reactions would be synthesized. If the plant is kept in the dark, on the other hand, then each substance, once exhausted, will not be synthesized again. This will make the whole process much easier to follow.)*
- Sketch a graph of expected concentration vs. time for substances A, B, C, and D. Put all the curves on the same graph. For a given substance, what shape would the graph have if carbon fixation were performed in the light? In the dark? *(Students should draw a series of bell-shaped curves, one after the other, representing A, B, C, and D, showing the concentration of each rising, peaking, and then dropping off as it is converted into the next intermediate, whose concentration would then rise, etc. Note that the curves should overlap at least somewhat; for example, A and B must be present at the same time if A is converted into B. If the reactions were carried out in an illuminated flask, then the concentration of each intermediate might increase until it reaches some steady concentration, where it is being generated at about the same rate as it is consumed.)*
- How could you stop the reaction at a specific time to study whatever mixture of intermediates is present? *(Kill the plants.)*
- Once you've obtained such a mixture of intermediates, how do you identify and separate specific molecules in it? *(Paper chromatography, similar to AP Bio Lab 4: Plant Pigments and Photosynthesis)*

Calvin and Benson set about solving the carbon fixation problem, a process that would take ten years and the full-time efforts of 10 Ph.D. students and 25 postdocs, as well as collaborations with visiting scientists from around the world—everywhere from Belgium to Japan to England. Calvin was responsible for the leadership and overall direction of the project, as well as the funding, and Benson worked out most of the technical details; for example, he developed special “lollipop” shaped flasks so that the algae they worked with could be evenly illuminated. First, the algae were exposed to light in the absence of CO_2; this isolated the products of the light reactions. Then, $^{14}CO_2$ would be introduced into the flask, and after a specified time interval, the algae would be dropped into alcohol to kill them. Finally, the products were separated by two-dimensional paper chromatography, which separated them along two axes based on their affinity for the different solvents used on the x- and y-axis. The volatile solvents smelled so awful that physicists working on the floor above the chromatography room, unused to the messy realities of experimental chemistry, often left the lab in disgust. The chromatography paper was applied to a piece of x-ray film, so that the location of the radioactive $^{14}CO_2$ (which darkened the paper) could be visualized:

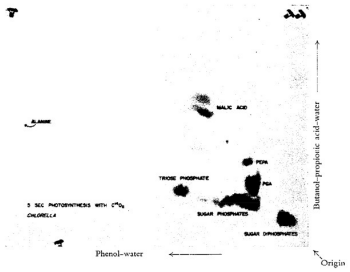


Fig. 6. Chromatogram of extract from *Chlorella* indicating uptake of radiocarbon during 5 seconds of photosynthesis.

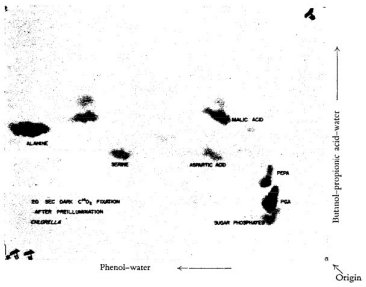


Fig. 7. Chromatogram of extract from *Chlorella* indicating uptake of radiocarbon, 20 seconds dark fixation after pre-illumination.

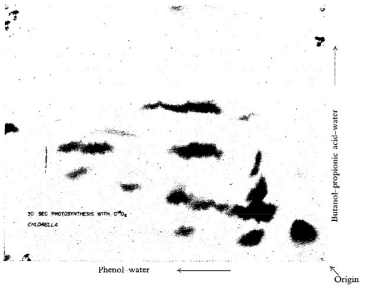


Fig. 4. Chromatogram of extract from algae indicating uptake of radiocarbon during 30 seconds of photosynthesis, using *Chlorella*.

Selecting precisely the right mixture of solvents to attain optimal separation of a particular mixture is a touchy task requiring much expertise. Benson selected the solvents and designed the reaction flasks, and Calvin obtained all the necessary funding and made the lab run like clockwork. By stopping the reaction at progressively longer intervals, they were able to map out the accumulation (and of the various products of photosynthesis. The interpretation of the chromatography paper, however, was far from straightforward. Spots on the paper were often ambiguous, and distinguishing one sugar phosphate from another often involved more than a little guesswork.

The experiment showed that a lot of 3-carbon molecules were produced immediately after the start of the reactions. How many carbon atoms do you think the initial carbon dioxide accepting molecule has? *(2)*

Early in the process, for example, the group searched (ultimately in vain) for a two-carbon CO_2 acceptor, reasonably predicting its existence because of the predominance of three-carbon intermediates early in the cycle. As it turned out, the initial acceptor was a five-carbon molecule, ribulose bisphosphate, which split into two three-carbon molecules (phosphoglycerates) almost immediately upon carboxylation. Occasionally, a spot would be so difficult to identify that the group would label it “godnose.” Analysis of the products was tedious, and involved many different procedures. Calvin described the process as “having only half of the data in hand, and half of it is wrong, but you don't know which half.” Tedious though it was, the approach worked, and the pieces gradually fell into place over the next ten years.

Thioctic acid

Eventually, with the steps of the cycle convincingly determined, Calvin's attention turned to the problem of the light reactions. Calvin had worked out a theory that came to be called the Thioctic Acid Theory. “It was the most exciting idea that I and many of my colleagues experienced,” Benson recalled in his memoirs, “and it was entirely Melvin's idea.” Thioctic acid (lipoic acid) had recently been discovered and characterized, and it was shown to be able to accept excited electrons from chlorophyll. The molecule's suspected metabolites (sulfur radicals) had been detected in abundance in photosynthetic tissues with the help of a newly invented spectrometer. Calvin predicted that the molecule's disulfide bond, located within a five-membered ring, should be susceptible to photolysis, and that the products of this reaction would react with water to generate (ultimately) the fuel for the carbon fixation cycle. Calvin, Benson, and the group worked “like beavers, day and night,” gathering data; Calvin also had help from chemists John Barltrop and John Brockman. Early data was convincing; treatment of algae with thioctic acid increased the rate of photosynthesis by 50%. Calvin's theory generated quite a bit of excitement. His work with Polanyi (on absorption and reemission of light by organic compounds) and Lewis was widely acknowledged to be an excellent background for

such a discovery. In 1954, Calvin presented his research to the American Association for the Advancement of Science. C.B. Van Niel, a prominent biochemist (described by Benson as “a god in bacterial metabolism”) responded with tears of joy, and congratulated Calvin on having “solved every problem in photosynthesis.” Further validation of Calvin's work would come from the isolation of thioctic acid from the chloroplast, which Benson set about doing:

> The assay was tedious and required microbiological experience. I grew some Chlorella in sulfate-S35, chromatographed the extract, and prepared the radio-autograph of my paper chromatogram. With Melvin and the others standing around the great white table, I laid the film on the paper. There was a huge black spot, right in the position we expected for thioctic acid. Melvin’s eyes just about dropped out onto the film. It was a breathtaking moment. The S35 radioactivity had to be proved to be thioctic acid.

If you were another scientist in attendance at the 1954 conference, how would you evaluate the importance of Calvin's work? Some things to consider:

-Evidence presented to support Calvin's theory
-Calvin's reputation in the scientific community
-Calvin's training, and the reputations of the chemists he worked with
-C.B. Van Niel's response

Calvin now had a critical piece of data that would confirm his theory, but it needed to be reproduced. They enlisted the help of Clint Fuller, a microbiologist working on photosynthesis, to use a sensitive bacterial assay to confirm the presence of thioctic acid. (Fuller, the first biologist to work in Calvin's lab full of chemists, helped improve the efficiency of their experiments when he discovered that their algae flasks were contaminated with yeast.) Fuller tried over and over again to isolate thioctic acid from chloroplasts, but could not. A closer look at the other pieces of evidence revealed errors there as well. Almost as soon as Calvin's theory of the light reactions was introduced, it was forgotten. In Calvin's 1989 memoir, he admitted that thioctic acid had turned out to be a “blind alley” for photosynthesis research. Nevertheless, Calvin had good theoretical reasons for advancing this line of inquiry, and his place in history was secured in spite of his pursuit of this blind alley.

The enzyme

Calvin's group was, according to science journalist Oliver Morton, “remarkably tolerant of errors and blind alleys”; when group members would discuss recent progress, Calvin would often seize upon any publishable result, however preliminary. (“The Path of Carbon in Photosynthesis” was published as a series of 23 papers.) More than once, a substance would be identified as a critical intermediate in one paper, only to have its importance downplayed in the next, and later on to disappear altogether; for example, paper #21 still referred to intermediates in the Thioctic Acid Theory. At the same time, Calvin was very much a hands-on manager. His colleagues often withheld information from him—sometimes because they were afraid he would go public prematurely, and sometimes so they'd still have something fresh when Calvin asked, “what's new?” as he would do almost every single day.

An experiment by Calvin and Massini in 1952 had identified ribulose bisphospate as the molecule to which CO_2 is initially affixed, but Calvin was content to move on without identifying the responsible enzyme. Calvin, an organic chemist, was not interested in enzymology—he saw it as a topic best left to the biochemists. Benson, though, took a keen interest in the question, and he recruited Fuller, who had valuable experience breaking cells an extracting enzymes; together with visiting Belgian scientist Jacques Mayaudon, Benson's group began to isolate and characterize the enzyme. They isolated it by putting spinach in a blender and using ammonium sulfate to precipitate out the

protein from the homogenate. Then, centrifugation was used to separate proteins from the mixture; fractions of the pellet were then re-dissolved, re-precipitated, and re-examined. Benson and Fuller used the microbial assay—the same test that had falsified Calvin's Thioctic Acid Theory—to measure enzyme activity. Benson and his colleagues carried on their work independently of Calvin, without his knowledge. It was never clear why they did this, but there were several possible reasons.

Why do you think Benson et al. worked on the enzyme project without involving Calvin?

One likely reason was Calvin's tendency to seize upon and go public with preliminary results; Benson, Mayaudon, Wildman, and Fuller may have been more cautious, more concerned with getting it right the first time. Another possibility was Calvin's lack of interest in the project; perhaps he would have been unwilling to support Benson's involvement if he knew the full extent of time and resources they were devoting to a research question Calvin felt was fundamentally uninteresting. After all, Calvin was in charge of fundraising, and probably felt strongly that he should have a say in what other scientists in his lab worked on. In an interview, Benson stated his view that Calvin was completely wrapped up in the Thioctic Acid Theory, to the exclusion of other work.

At Caltech, Sam Wildman was taking the opposite approach—instead of working to isolate the enzyme of interest from cells, he instead removed everything from a cell extract that wasn't a protein, and characterized what was left over. As it turned out, one particular protein comprised the vast majority of the first fraction to emerge after centrifugation; Wildman called it “fraction 1 protein.” Benson's in-laws lived in Pasadena, so he found himself at Caltech frequently, and he and Wildman compared notes. As it turned out, the same series of steps that yielded “carboxydismutase” (as the enzyme was initially called) in Benson's experiments also yielded “fraction 1 protein” in Wildman's. Benson's bottom-up approach and Wildman's top-down approach seemed to be converging on the same protein.

Do Benson, Wildman, Fuller, and Mayaudon have good evidence to support their claim of having discovered the enzyme that catalyzes carbon fixation? Consider the following: -the nature of collaboration -agreement between multiple lines of evidence -reproducibility of results

Benson later recalled, in his memoirs, “I consider our discovery that the enzyme catalyzing carboxylation of ribulose diphosphate was the same predominant protein isolated by Sam Wildman one of my most exciting revelations. Forty-six years later, Sam Wildman still recalls my phone call with the news.” The project had literally come full circle; the all-important carbon fixation step could now be studied in terms of the enzyme that catalyzed it. (The kinetics of rubisco, as ribulose bisphosphate carboxylase is usually shortened to today, are a major factor in photorespiration and alternative photosynthetic pathways, and a major object of interest to genetic engineers.)

Benson's Dismissal

In 1954, Benson excitedly wrote up his findings and sent the paper to Calvin. (This step was unavoidable; since the Old Radiation Laboratory was a government lab, regulations required principal investigators to submit all pending publications to a committee for in-house peer review.) At first, Calvin ignored the paper; he was busy with the Thioctic Acid Theory, and Benson speculated that Calvin didn't understand the paper or its importance. The paper languished for three years, but was

eventually submitted and published, either by Calvin or his secretary. By 1957, the discovery had already been replicated elsewhere, and was hardly the breakthrough it would have been if it were published in 1954. The paper was published with Mayaudon's name, but not Benson's.

Should scientists be bold or conservative? (Consider the motivations of scientists who would make this decision.) Do you think papers published by “bold” scientists like Calvin would have important differences with papers published by “conservative” scientists like Benson? What might have happened if Benson had instead bypassed the established review process and went public directly with his discovery of rubisco?

Calvin became furious that Benson was doing work behind his back and fired him. Afterwards, none of the ORL papers were published with Benson's name on them, and Calvin would not help him find work. Calvin made no mention of Benson in his autobiography, *Following the Trail of Light*, though he did in his Nobel lecture and in his 1989 memoir in *Photosynthesis Research*.

Why did Calvin fire Benson? Was it a result of a strong theoretical disagreement, or Calvin's ego? Was he frustrated with the success of Benson's experiments, while the Thioctic Acid Theory failed? Was it business, or personal, or both? How can you tell?

Aftermath

The path of carbon in photosynthesis has come to be called the Calvin cycle, and Calvin alone was ultimately recognized with a Nobel prize. What work went into the discovery? How did the various scientists involved depend on each other's work? Does any one individual deserve recognition for the discovery? If you had been on the Nobel committee, who would you have selected to receive the prize?

Melvin Calvin went on the receive, alone, the Nobel Prize for Chemistry in 1963.