Photosynthesis Research **80:** 77–83, 2004.

Tribute

Samuel Ruben's contributions to research on photosynthesis and bacterial metabolism with radioactive carbon

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Received 1 April 2003; accepted in revised form 10 August 2003

Key words: Niels Bohr, ^{14}C, CO_2 fixation, George de Hevesy, Howard Gest, Zev Hassid, Martin Kamen, E.O. Lawrence, photosynthesis, phosphorylation, Samuel Ruben

Abstract

The earliest experiments on the pathways of carbon in photosynthetic and heterotrophic metabolism using radioactive carbon, ^{11}C, as a tracer were performed by Samuel (Sam) Ruben, Martin Kamen, and their colleagues. The short half-life of ^{11}C (20 min), however, posed severe limitations on identification of metabolic intermediates, and this was a major stimulus to search for a radioactive carbon isotope of longer half-life. ^{14}C was discovered by Ruben and Kamen in 1940, but circumstances prevented continuation of their research using the long-lived isotope. Because of the untimely accidental death of Ruben in 1943, there are very few published accounts on the life and work of this extraordinary scientist. This paper summarizes highlights of Ruben's outstanding accomplishments.

> 'It is a noble employment to rescue from oblivion those who deserve to be remembered.'
> – Pliny, the Younger (A.D. ca. 62–113), in a letter to Titinius Capito

The discovery of ^{14}C

The discovery of long-lived ^{14}C by Samuel Ruben (1913–1943) and Martin Kamen (1913–2002) in 1940 was a momentous event for the future of research in biochemistry. At the time, Sam Ruben and Martin Kamen were 27 years old, working in different departments at the University of California, Berkeley. Ruben was an instructor in the Department of Chemistry, and Kamen was a 'research fellow' in the Radiation Laboratory (headed by Ernest O. Lawrence, inventor of the cyclotron).

After the discovery of ^{14}C, Ruben became associated with a National Defense Research Committee (NDRC) project concerned with chemical warfare research. This involved measuring the ambient concentrations of gases, including phosgene, released into the atmosphere. On 27 September 1943, during the course of a laboratory experiment with a defective ampoule containing liquid phosgene, Ruben inhaled a large dose of the toxic gas and died the next day. The fatal accident, described in detail by Kamen (1986a), Benson (2002) and Johnston (2003) occurred 1 month after Ruben was promoted to the rank of Assistant Professor.

Because of his premature death, relatively little has been written about Ruben's life. Since I became Kamen's first graduate student only six years after the discovery of ^{14}C, I have had a long interest in Ruben's short, but meteoric, career relating to photosynthesis and other aspects of biochemistry. Recently, I acquired new insights into the first uses of radioactive carbon (and ^{32}P) in biochemical research at Berkeley; mainly from two sources: (a) examination of documents in the Ruben/Kamen File in the Bancroft Library, University of California, Berkeley, and (b) a serendipitous meeting with Professor Harold Johnston, Department of Chemistry, University of California, Berkeley.

Professor Johnston recently published a book (Johnston 2003) on secret chemical warfare research at CalTech and Berkeley during World War II. Chapter 3 consists of a 'brief biography of a remarkable scientist, Samuel Ruben.'

Backgrounds of Ruben and Kamen

Ruben and Kamen had significant parallels in their backgrounds. To begin with, both were born in 1913. Ruben's parents emigrated to the United States, from Poland, in 1910. Kamen's father was born in 'White Russia' (Belorusskayo) and his mother 'somewhere in Lithuania or Latvia'; they met in Toronto (Canada) and came to the United States in 1911. Kamen's autobiography (1986a) details his family background, his education and career (for a condensed informative summary, see Kauffman 2000). Johnston (2003) summarizes a comparable description of Ruben's life.

Kamen received a PhD degree in Chemistry from the University of Chicago in 1936. His research was on proton–neutron interactions and his dissertation was published as a paper in the Physical Review by Harkins et al. (1936). Kamen (1986a) noted the paper 'was accepted as a doctoral dissertation after the formality of printing a new title page naming me as sole author and binding it as a reprint' Ruben's PhD degree, from the University of California in 1938, also was in chemistry, but included biological experiments with radioactive phosphorus as a tracer (see later).

How did the Ruben–Kamen 'team' come together?

In late December 1936, Kamen traveled to the west coast to visit Berkeley. He and Ruben soon joined forces as the result of 'correction of a mistake made by E.O. Lawrence' (Johnston 2003):

> James Cork, a visiting professor of physics working with Ernest Lawrence in 1937, passed a beam of high energy deuterons through a stack of thin platinum foils and found some unexpected and thus interesting results. When Lawrence proudly showed these results to the great physicist Niels Bohr during his visit to Berkeley, Bohr remarked that he did not believe them. Bohr's knowledge and intelligence were so high that Lawrence did not argue with him, but later asked the young physicist Ed McMillan [Nobel-laureate 1951] to review the problem. McMillan saw the experiment involved both physics and chemistry. He selected Martin Kamen, a recently arrived visitor, to be the physicist to redo the experiments, and he asked Dean Wendell Latimer of the College of Chemistry to help him find a trusted chemist to work on the problem. Latimer recommended the graduate student Sam Ruben, and so the highly productive pair of Kamen and Ruben came together.... After a lengthy investigation, Kamen and Ruben found that the previous results were spurious, the result of trace amounts of contaminants, which gave strong signals when exposed to the neutrons, which were a by-product of the beam. Lawrence accepted and appreciated the work Kamen and Ruben had done, and invited Kamen to join the staff as a Research Fellow of the Radiation Laboratory at a salary of one hundred dollars per month, which Martin was delighted to accept. His assigned job was to use the cyclotrons to make radioactive substances to be used as tracers for experiments at Berkeley and elsewhere.

The hard work involved in explaining the platinum–deuteron results drew Kamen and Ruben together in a relationship that developed into a strong friendship. 'We decided to form a partnership in which the facilities of both the Rad Lab and the Chemistry Department could be exploited to the maximum' (Kamen 1986a). They reached an understanding that whatever concerned production and characterization of isotopes produced in the cyclotron would be Kamen's responsibility, while their application in chemical research would be Ruben's.

A dramatic public announcement by Professor R.T. Birge

'Radioactive carbon of long half-life' was the title of the first brief account of the discovery of ^{14}C (Ruben and Kamen 1940), which was soon followed by a complete description (Ruben and Kamen 1941). In the preliminary note, the authors predicted that 'long-lived radio-carbon will be of great importance for many chemical, biological, and industrial experiments.' Within a few days after Ruben and Kamen convinced themselves that the 'new' radioactivity was indeed an isotope of carbon, the discovery of ^{14}C was made public during the presentation of the 1939 Nobel Prize in physics to Lawrence. Based on Kamen's (1986a) detailed description of events during February 1940, Johnston (2003) summarized the 'public birth' of ^{14}C as follows:

> On February 29, 1940, there was a momentous ceremony in Wheeler Hall [University of California at Berkeley], where the Swedish Consul presented the Nobel Prize to E.O.L.... The chairman of the Physics Department, R.T. Birge, gave the presentation address. He spoke of the great importance of radioactive isotopes as tracers in biology and possibly as therapeutic agents. Then in a dramatic gesture wholly atypical of him, he stepped back, raised his arm, and portentously announced, 'I now have the privilege of making a first announcement of very great importance. This news is less than twenty-four hours old and hence is real news. Now, Dr S. Ruben, instructor in chemistry, and Dr M.D. Kamen, research associate in the Radiation Laboratory, have found by means of the cyclotron, a new radioactive form of carbon, probably of mass fourteen and average life of the order of magnitude of several years. On the basis of its potential usefulness, this is certainly much the most important radioactive substance that has yet been created.'

Details of the discovery of ^{14}C have been thoroughly documented, mainly in publications of Kamen (1963, 1986a).

The Ruben/Kamen file in the Bancroft Library

It is of interest that one of the first entries in Kamen's Rad Lab I notebook (bound) is a lengthy, detailed account of a lecture on nuclear physics given by Niels Bohr at Berkeley on 18 March 1937. Figure 1 shows part of the first page of Kamen's notes, which are typical of his small script and clear handwriting. In contrast, records of Ruben and Kamen's early experiments using radioactive carbon in biological experiments are in rough handwriting, on loose sheets of paper. Records of particular experiments are held together by paper clips, and are what I would call 'barebones protocols'... terse descriptions of conditions and experimental setups. The results of radioactivity counts are detailed. Otherwise, the descriptions are sketchy, with little discussion and conclusions given in just a few words. From my long experience with Kamen's handwriting, I conclude that these records must have been made by Ruben, and in a hurried fashion. In many instances, the month and day of the experiment are given, but not the year.

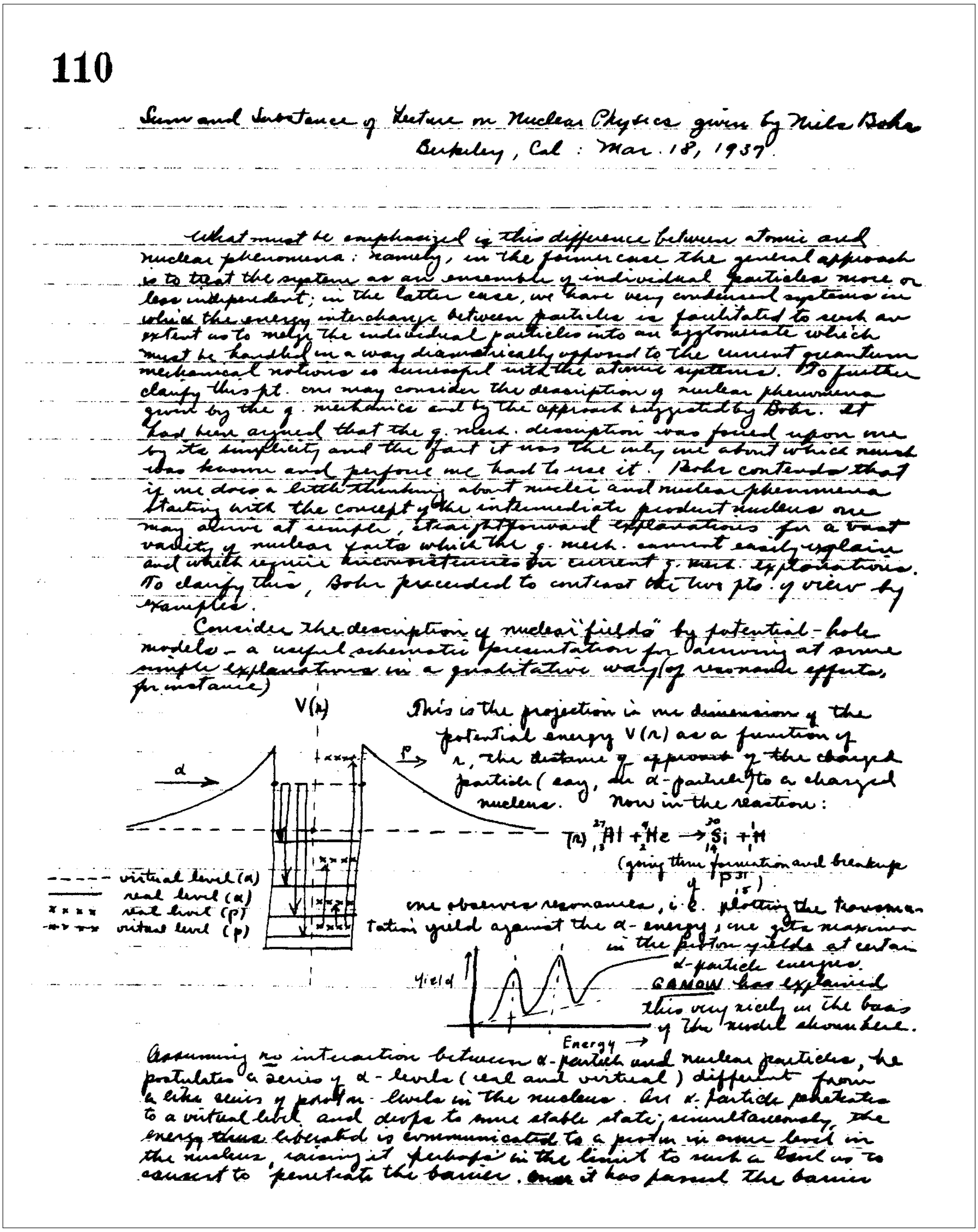
The majority of the items in the file appear to have belonged to Ruben. These include published reprints on a very wide variety of subjects, biological and chemical, which reflect Ruben's early and wide interest in biochemistry. This is also indicated by sophisticated correspondence with scientists on photosynthesis and other topics. Such interests were foreshadowed in Ruben's PhD dissertation, which consisted of three sections: fundamental physics of an iodine resonance neutron bond; use of radioactive iron to study exchange between Fe^{3+} and $Fe(CN)_6^{3-}$; radioactive phosphorus as an 'indicator' in phospholipid metabolism.

The last section of Ruben's dissertation describes experiments done very soon after George de Hevesy, a pioneering chemist, extended to biological systems his concept of using radioactive isotopes as 'indicators' [i.e., *tracers*] for studying the dynamics of chemical processes. In 1937, Hevesy and colleagues published a number of papers on the use of ^{32}P in experiments with plants and animals. Ruben was very quickly familiar with this research; during 1937 and 1938 he was a coauthor of four papers on 'Radioactive phosphorus as an indicator of phospholipide metabolism' (see, for example, Perlman et al. 1937). Hevesy was awarded the Nobel Prize for chemistry in 1943, the year Ruben died. Hevesy's biographer (Levi 1985) noted that when he began his biological work, 'his knowledge in the fields of biology and biochemistry was scanty, to say the least.' But he had the same quality that Ruben had . . . he learned new fields quickly.

Ruben, the driving force of the biological experiments

It is very clear from Kamen's autobiography (1986a) that Ruben was responsible for the decision to work on photosynthesis:

> I should emphasize that the Department of Chemistry held the university biologists and their work in low esteem and it took extraordinary courage on the part of a young instructor such as Sam to commit himself to a program in biological research. The prospects of such research providing a basis for promotion in the department were exceedingly dim. Nevertheless, Sam persisted, and his doing so eventually aided the creation of many great research laboratories on the Berkeley cam-



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Sum and Substance of Lecture on Nuclear Physics given by Niels Bohr
Berkeley, Cal: Mar. 18, 1937.

What must be emphasized is this difference between atomic and nuclear phenomena: namely, in the former case the general approach is to treat the system as an ensemble of individual particles more or less independent; in the latter case, we have very condensed systems in which the energy interchange between particles is facilitated to such an extent as to merge the individual particles into an agglomerate which must be handled in a way diametrically opposed to the current quantum mechanical notions so successful with the atomic systems. To further clarify this pt. one may consider the description of nuclear phenomena given by the q. mechanics and by the approach suggested by Bohr. It had been argued that the q. mech. description was forced upon one by its simplicity and the fact it was the only one about which much was known and perforce one had to use it. Bohr contends that if one does a little thinking about nuclei and nuclear phenomena starting with the concept of the intermediate product nucleus one may arrive at simple, straightforward explanations for a vast variety of nuclear facts which the q. mech. cannot easily explain and which require inconsistencies in current q. mech. explanations. To clarify this, Bohr proceeded to contrast the two pts. of view by examples.

Consider the description of nuclear "fields" by potential-hole models – a useful schematic presentation for arriving at some simple explanations in a qualitative way (of resonance effects, for instance)

This is the projection in one dimension of the potential energy V(r) as a function of r, the distance of approach of the charged particle (say, an α-particle) to a charged nucleus. Now in the reaction:

[illegible] $^{27}_{13}Al + ^{4}_{2}He \rightarrow ^{30}_{14}Si + ^{1}_{1}H$

(going thru formation and breakup of P^{31}_{15}).

one observes resonances, i.e. plotting the transmutation yield against the α-energy, one gets maxima in the proton yields at certain α-particle energies. GAMOW has explained this very nicely on the basis of the model shown here.

Assuming no interaction between α-particle and nuclear particles, he postulates a series of α-levels (real and virtual) different from a like series of proton-levels in the nucleus. An α-particle penetrates to a virtual level and drops to some stable state; simultaneously, the energy thus liberated is communicated to a proton in some level in the nucleus, raising it perhaps in the limit to such a level as to cause it to 'penetrate the barrier'. Once it has passed the barrier

Figure 1. 'Sum and substance of lecture on nuclear physics given by Niels Bohr, Berkeley, California: March 18, 1937.' Notes (first page) by Martin Kamen, reproduced by courtesy of the Bancroft Library, University of California, Berkeley.

pus dedicated to tracer research in basic biology and medicine. Sam had read the standard textbook exposition of green plant photosynthesis as a process in which CO_2 and water combined to produce glucose and oxygen. He reasoned that if radioactive CO_2 was administered to a green plant in the

Figure 2. Samuel Ruben (left) and Zev Hassid (right); late 1930s or early 1940s; photograph reproduced by courtesy of Dr George C. Ruben, Dartmouth College. During the summer of 1947, the author (HG) visited Hassid in Berkeley to learn how to prepare ^{14}C-labeled radioactive glucose from plant leaves that had been exposed to $^{14}CO_2$ and light.

> light, it would incorporate the radioactive 'label' in all the six carbons of glucose. The use of such labeled glucose would make it possible to determine for the first time how animals utilized sugars in their metabolism.

Prior to the discovery of ^{14}C, Ruben, Kamen, the plant biochemist Zev Hassid, and Don C. DeVault (see Figure 2 for a photograph of Ruben and Hassid) used ^{11}C as a tracer (Ruben et al. 1939). Because of its short 20 min half-life, experiments had to be done very quickly and rapid isolation of labeled metabolic intermediates was hectic as well as inherently problematic (Benson 1977, 2002). Ruben and Kamen experienced various difficulties in preparing radioactive glucose for experiments with rats, and this led Ruben to a revelation (Kamen 1986a, p. 84):

> During a recital of these troubles, Sam suddenly stopped, his eyes widened, and he blurted 'Why are we bothering by the rats at all? Hell, with you and me together we could solve photosynthesis in no time!' From that moment, we were out of everything but the photosynthesis business. I was just as excited as Sam at the prospect of solving the Big Problem – identifying at long last the initial product of CO_2 fixation in green plant photosynthesis – a mystery that had plagued chemists and biologists ever since the original discoveries of Joseph Priestley, Théodore de Saussure, Jan Ingen-Housz, Jean Senebier, and others in the eighteenth century and thereafter.

Carbon-14 in 1946/1947

During World War II, I was a member of a unit of the Manhattan Atomic Bomb Project responsible for characterization of the numerous radioactive isotopes created by the fission of uranium (Gest 1994, 2001). Soon after the war ended, I resumed graduate work with Kamen at Washington University, who at the time was studying CO_2 reduction by unicellular green algae. In 1946, we had available only a very small quantity of $Ba^{14}CO_3$, obtained from the Manhattan Project Isotope Research Division. This was used to study 'Differential inhibition of respiration and dark CO_2-fixation in *Scenedesmus* and *Chlorella*' (Allen et al. 1947). While experiments were being conducted during the summer of 1946, Kamen was at Cold Spring Harbor writing his seminal book 'Radioactive Tracers in Biology' (Kamen 1947). On August 9, Kamen wrote me:

> The book is progressing. I am 2.5 chapters from the end of the first draft. I expect it to be finished in the next two weeks. I will then be ready to crawl back to St. Louis and die. There will be at least a month of tightening the style, checking references, indexing, preparing a glossary of terms etc. I will get drunk one night and write the apologia or preface.

Back in hot St. Louis, I had my hands full. My first child, Theodore, was born on 29 July 1946. In addition to the experiments on algae, I was processing a large batch of ^{32}P, produced in the Washington University cyclotron, which had to be purified for use by clinicians at the School of Medicine (where our laboratory was located). On August 14, I wrote Kamen:

> The C-14 finally arrived. . .an Oak Ridger on the tracer preparation staff, Ralph Overman, was in to see me yesterday. He offered to be of service in expediting our needs, etc. He says their measurements and those of Norris indicate the approx. 6000 year half-life.

In their classic paper on ^{14}C, Ruben and Kamen (1941) estimated that the half-life was 'probably 10^3–10^5 years.' Eventually, it was established to be 5700 years.

In his 1947 book, Kamen mentioned that 'The Manhattan Project has announced availability of radioactive samples assaying 1–10% ^{14}C. . . . The cessation of the ^{14}C supply during the war years, which ensued almost immediately after its discovery, accounts for the almost non-existent literature concerned with its application.' At the time, the only published research using this isotope concerned fatty acid metabolism in two species of bacteria. Summarizing the use of ^{14}C in metabolic research, Fruton (1972) noted that although the isotope had been prepared by Ruben and Kamen (1940),

> . . . it did not become generally available for metabolic studies until after World War II, when the cyclotron was replaced by the nuclear reactor ('atomic pile') as the source of artificial radioisotopes. Within about ten years (1945–1955), the use of ^{14}C as a metabolic tracer resolved many of the questions that been hotly debated for a half-century, and elucidated many unknown pathways of intermediary metabolism.

Epilogue

A careful reading of Kamen (1986a), Benson (2002) and Johnston (2003) reveals Ruben's dynamic character, charisma, great energy, versatility and fearless entry into biological research problems in which he had little or no previous training. Kamen (1963) summarized Ruben's influence and abilities as follows:

> Ruben was responsible, almost single-handedly, for the growth of interest in tracer methodology which occurred at Berkeley in the years 1937–1938. His unique combination of experimental skills, energy, wide-ranging interests, and quick grasp of essentials when confronted with new and unfamiliar areas of science, provided a focus for the efforts of an ever-increasing number of able investigators.

Ruben's bibliography consists of about 26 papers. His publications during 1940, the year ^{14}C was discovered, are listed below, alphabetically, and testify to his extraordinary ability and productivity. (See Johnston (2003) for a more complete list.)

> Barker HA, Ruben S and Kamen MD (1940a) The reduction of radioactive carbon dioxide by methane-producing bacteria. Proc Natl Acad Sci USA 26: 426–430
>
> Barker HA, Ruben S and Beck JV (1940b) Radioactive carbon as an indicator of carbon dioxide reduction. IV. The synthesis of acetic acid from carbon dioxide by *Clostridium acidi-urici*. Proc Natl Acad Sci USA 26: 477–482
>
> Carson SF and Ruben S (1940) CO_2 assimilation by propionic acid bacteria studied by use of radioactive carbon. Proc Natl Acad Sci USA 26: 422–426
>
> Ruben S and Kamen MD (1940) Radioactive carbon in the study of respiration in heterotrophic systems. Proc Natl Acad Sci USA 26: 418–422
>
> Ruben S, Kamen MD and Hassid WZ (1940a) Photosynthesis with radioactive carbon. II. Chemical properties of the intermediates. J Am Chem Soc 62: 3443–3450
>
> Ruben S, Hassid WZ and Kamen MD (1940b) Radioactive nitrogen in the study of N_2 fixation by non-leguminous plants. Science 91: 578–579
>
> Overstreet R, Ruben S and Hassid WZ (1940) The absorption of bicarbonate ion by barley plants, as indicated by studies with radioactive carbon. Proc Natl Acad Sci USA 26: 688–695

Ruben's last paper, published the year of his death, was entitled 'Photosynthesis and phosphorylation' (Ruben 1943). The last paragraph of the summary:

> A new formulation of the mechanism of photosynthesis is briefly presented which offers a plausible model for the fixation and reduction of carbon dioxide not only for green plant photosynthesis but also for carbon dioxide fixation and reduction by the many different chemosynthetic and heterotrophic organisms.

In this paper, Ruben developed a sophisticated analysis of the bioenergetics of photosynthetic metabolism. His scheme for conversion of radiant energy to chemical energy invoked a 'coupled oxido-reduction reaction to form high energy phosphate donors,' anticipating the experimental demonstrations of photophosphorylation a decade later by Frenkel (1954) and Arnon et al. (1954).

For reviews of the history of research on the roles of phosphorylation in photosynthesis metabolism, see Gest and Kamen (1948) and Gest (1993, 2002). Papers by Kamen (1986b, 1989) provide additional perspectives, and Jagendorf (2002) has discussed, in these history issues, the history of photophosphorylation and the chemiosmotic hypothesis.

Acknowledgments

I am indebted to Professor George C. Ruben, Dartmouth College, for photographs and information about his father, Samuel Ruben. I also thank Professor Harold Johnston, University of California (Berkeley) for providing me with Chapter 3, a short biography of S. Ruben, from his forthcoming book ('A Bridge Not Attacked'). Archivist David Farrell, Bancroft Library (Berkeley) generously arranged access to the Ruben/Kamen files, and Roger Beckman, Life Sciences Library, Indiana University (Bloomington) assisted with skillful electronic searches of the literature.

This paper was edited by J. Thomas Beatty and Govindjee.

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Fruton JS (1972) Molecules and Life/Historical Essays on the Interplay of Chemistry and Biology. Wiley Interscience, New York

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