Photosynth Res (2017) 134:93–105
DOI 10.1007/s11120-017-0410-y

TRIBUTE

The paths of Andrew A. Benson: a radio-autobiography

Arthur M. Nonomura[1] · **Barry Holtz**[2] · **Karl Y. Biel**[3] · **Robert Cooney**[4] · **George Lorimer**[5] · **Govindjee**[6]

Accepted: 29 May 2017 / Published online: 3 July 2017

Abstract Andrew A. Benson, one of the greatest *biochemists* of our time, is celebrated on his centennial by the authors with whom he interacted performing experiments or contemplating metabolic pathways in a wide range of biological kingdoms. He charted the chemical flow of energy in cells, tissues, organs, plants, animals, and ecosystems. Benson collaborated with hundreds of colleagues to examine the natural history of autotrophy, mixotrophy, and heterotrophy while elucidating metabolic pathways. We present here a biological perspective of his body of studies. Benson lived from September 24, 1917, to January 16, 2015. Out of over 1000 autoradiograms he produced in his life, he left a legacy of 50 labeled autoradiograms to the authors who tell the story of his life's work that resulted in Benson's Protocol (Nonomura et al., Photosynth Res 127:369–378, 2016) that has been applied, over the years, for the elucidation of major metabolic pathways by many scientists.

Keywords Carbon reactions of photosynthesis · Golden age of metabolic biology · Radioisotope

The time of the publication of this Tribute coincides with what would have been the 100th birthday of Andrew (Andy) A. Benson, who was born on September 24, 1917, and passed away on January 16, 2015. We celebrate Andy's life by laying this final stepping-stone in *The Path of Carbon in Photosynthesis*, C. We use the Roman numeral for 100, which is also the notation for the element carbon. William W. Adams III (of the University of Colorado at Boulder) has edited this Tribute to Benson; it was reviewed by Hartmut Lichtenthaler and an anonymous reviewer. On the basis of these two reviews and the suggestions of William Adams, the revised version of this paper was approved for publication on the *Memorial Day*, by Thomas D. Sharkey (Editor, Photosynthesis Research), who added: "This tribute on the occasion of the 100th anniversary of Benson's birth goes well beyond describing his seminal and essential role in elucidating the path of carbon in photosynthesis. Readers will become more aware that Benson was a visionary who, directly or indirectly, helped elucidate many biochemical pathways by his use of many radioactive tracers in many biological systems. The insights by the authors of the tribute, who knew Benson personally, add significantly to our understanding of this biochemical pioneer."

Electronic supplementary material The online version of this article (doi:10.1007/s11120-017-0410-y) contains supplementary material, which is available to authorized users.

✉ Arthur M. Nonomura
Art.N@BRANDTiHammer.co

Barry Holtz
rbholtz@gmail.com

Karl Y. Biel
karlbiel@hotmail.com

Robert Cooney
rvcooney@hawaii.edu

George Lorimer
glorimer@umd.edu

Govindjee
gov@illinois.edu

1 Carbon Reactions of Photosynthesis Sector, Brandt iHammer, 479 Village Park Drive, Powell, OH 43065, USA

2 Holtz Biopharma Consulting, Bryan, TX 77807, USA

3 Institute of Basic Biological Problems, Russian Academy of Sciences, Pushchino, Moscow Region, Russia 142290

4 Department of Public Health Services, University of Hawaii at Manoa, Honolulu, HI 96822, USA

5 Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742, USA

6 Department of Plant Biology, Department of Biochemistry, and Center of Biophysics & Quantitative Biology, University of Illinois at Urbana-Champaign, 265 Morrill Hall, 505 S. Goodwin Avenue, Urbana, IL 61801, USA

Introduction

Andrew A. Benson has been called "*The Atomic Man*" for his bold biomolecular explorations utilizing radiolabeled chemicals during the nuclear era of the 1940s—his concepts set the course for the discoveries of many metabolic pathways based on his eponymous protocol (see G. Lorimer in Nonomura et al. (2016) for *Benson's Protocol*). Andy, indeed, developed the first protocols for peaceful uses of radioisotopes in many biological applications. His science has been hailed as elegant (Fomina and Biel 2016; Buchanan et al. 2016) and superb (Lichtenthaler et al. 2015). Initially, Andy called his radioactive-label tracer technique radio-autography, but this is now generally called *autoradiography*. Be that as it may, Andy's legacy of over a thousand autoradiograms leads us to use this radio-auto**bio**graphy to commemorate his life's work. Thus, we will let his autoradiograms tell the story of historical achievements that were the stuff of Andy's rigorous science and through which he opened enduring vistas (Buchanan et al. 2007; Lichtenthaler et al. 2008; Buchanan and Douce 2015). Andy spent over half a century at Hubbs Hall, Scripps Institution of Oceanography (see Fig. 1 for a 2007 photograph taken outside his office on the 4th floor balcony). To complete Benson's story, we show here (and in the Supplementary Material; also see Online Appendix) a sample of the 50 autoradiograms he left with us, many of which have never been published.

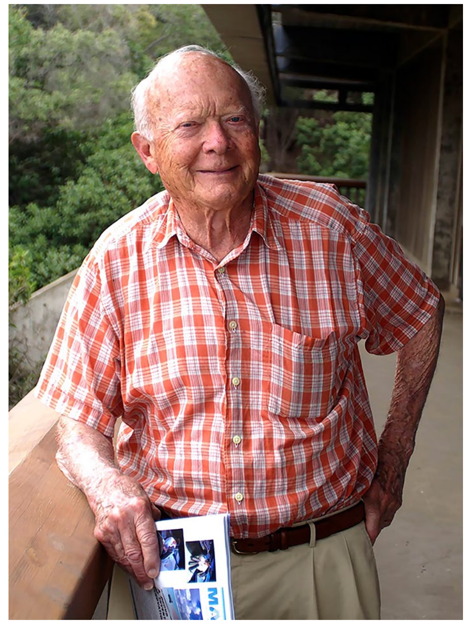

Fig. 1 A 2007 photograph of Andrew Alm Benson. He was on the balcony outside his office in Hubbs Hall, Scripps Institution of Oceanography, La Jolla, California USA. From the Image galleries, https://scripps.ucsd.edu/news/obituary-notice-andrew-benson-world-renowned-scripps-plant-biochemist; with the permission of Mario Aquilera, Scripps Communications Office, Scripps Institution of Oceanography; image by Arthur Nonomura

Charting the path

To address the question "*How does one see the carbon*?" Andy tracked the path of carbon metabolism by his treatment of plants and algae with accumulated concentrates of $^{14}CO_2$. The radiation emitted from metabolites that were tagged with ^{14}C could be captured as black spots and trails on X-ray films and, thus, be readily viewed. This process of developing X-ray film that was exposed to two-dimensional (2D) paper chromatograms of radiolabeled metabolites is *autoradiography*. Andy's materials included Whatman No. 1 filter paper, which was as large as Kodak Photoradiography Medical X-Ray Film SB-54—single coated blue sensitive tinted Estar Safety Base, with interleaved rounded corner, 14 × 17 inch sheets (see Benson et al. 1950). Starting in 1946, Andy built the laboratory he worked in, ordered instrumentation to his specifications, and grew large volumes of algal cultures. Figure 2 shows Andy with a row of 1.8 L Fernbach flasks, illuminated with fluorescent light panels. He initially used dense cultures of *Chlorella ellipsoidea* or *Scenedesmus obliquus*, pouring them into special glass vessels (called "*lollipops*"; see Fig. 12 in Nonomura and Benson 2013), then adding $^{14}CO_2$ and providing illumination. At specific intervals, he quickly dropped the entire contents of the lollipop into hot methanol to instantly stop the reactions at different steps of the *Path to Carbon*

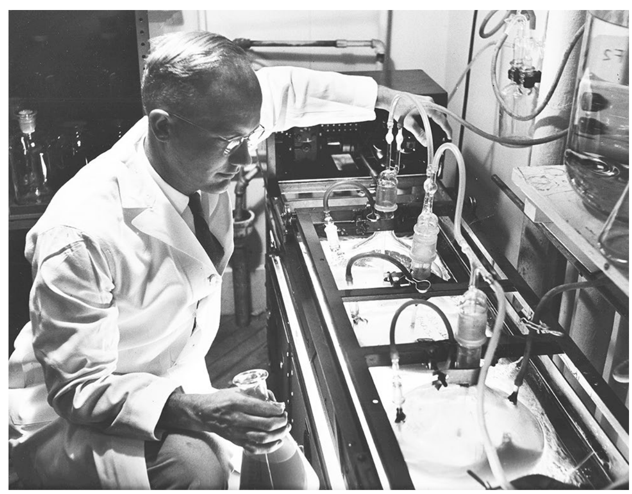

Fig. 2 Andrew A. Benson with his microalgal culture system. Pure cultures of *Chlorella* or *Scenedesmus* were grown in illuminated Fernbach flasks. After reaching a high cell density, cell suspensions were transferred to a lollipop for irradiation. From the private collection of Dee Benson

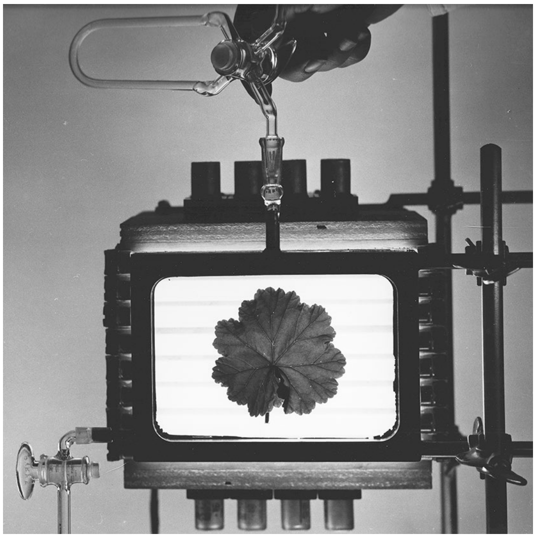

Fig. 3 Advanced generation *lollipop* designed for treatment of whole leaves with radioisotopes; inside, a geranium leaf. Both glass faces were illuminated during an experimental run. See Nonomura and Benson (2013) for an overview of a glass lollipop. From the private collection of Dee Benson

Fixation. Andy also grew higher plants and designed modified lollipops for radioisotope uptake by whole leaves. Figure 3 shows such a lollipop with a geranium leaf installed inside.

Andy, a highly capable chemist, synthesized radiolabeled compounds, developed chromatographic innovations, and initiated autoradiography. Within 4 years, Andy had not only tracked $^{14}CO_2$ to its early metabolites following carboxylation in photosynthesis, but he had also utilized ^{32}P to track its metabolism to phosphate esters. Figure 4a shows an autoradiogram of a soybean leaf hydrolysate of phosphorylated compounds, where the chromatographic spots could not be identified, underscoring the difficulty and complexity involved in identifying these compounds. As an outcome of studying with Andy, Alex Wilson identified comparable spots in *Scenedesmus* soon thereafter (see Buchanan et al. 1952). Figure 4b shows an autoradiogram of a marine red alga *Porphyra umbilicalis*, where the chromatographic spots were identified (see Fig. 5 in Govindjee 2010, showing Andy holding a labeled autoradiogram of *Scenedesmus* that was fed with $^{14}CO_2$). For this purpose, Andy generated a number of keys that showed the positions of numerous compounds that he had meticulously and consistently chromatographed in 2D (for one of the keys, see Fig. 5). For Andy's outstanding contributions to the elucidation of the carbon reduction cycle in photosynthesis, made primarily through his development of double labeling techniques employing ^{14}C, ^{3}H, and ^{32}P, *The Ernest Orlando Lawrence Award* was conferred on him. This award read: "Laureate Andrew A. Benson, 1962, Life Sciences" (see DOE 1962) and was accompanied by a check for $20,000. We suggest that without Andy's early creative approach, mentioned above, ribulose bis phosphate (RuBP; Ru1,5 DP in Fig. 5) would not have been identified then as the key intermediate in the carbon reactions of photosynthesis.

Bold explorations

We ascribe the breadth of Andy's research to his adventurous character. See, for example, investigations on lobsters by Cooney and Benson (1980). One of us (Arthur Nonomura) remembers Andy actively monitoring field research among thousands of acres of thorny roses (see Fig. 6). As

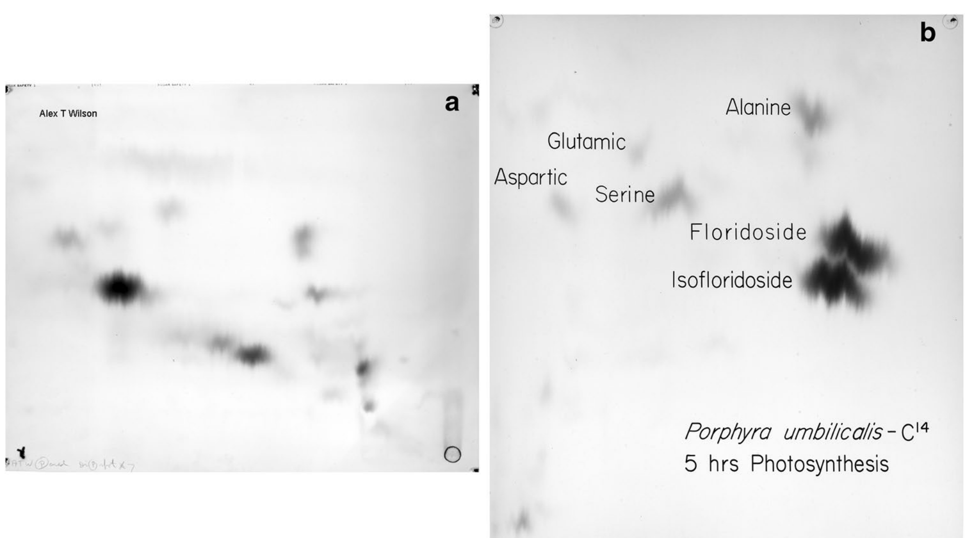

Fig. 4 **a** Autoradiogram by Alex T. Wilson (Buchanan et al. 1952; ATW was a coauthor). The soybean leaf hydrolysate of phosphorylated compounds was not labeled and underscores the difficulty and complexity involved in identifying the compounds. **b** Previously unpublished autoradiogram by A. A. Benson; it shows locations of identified compounds after 5 h of $^{14}CO_2$ photosynthesis by *Porphyra umbilicalis*

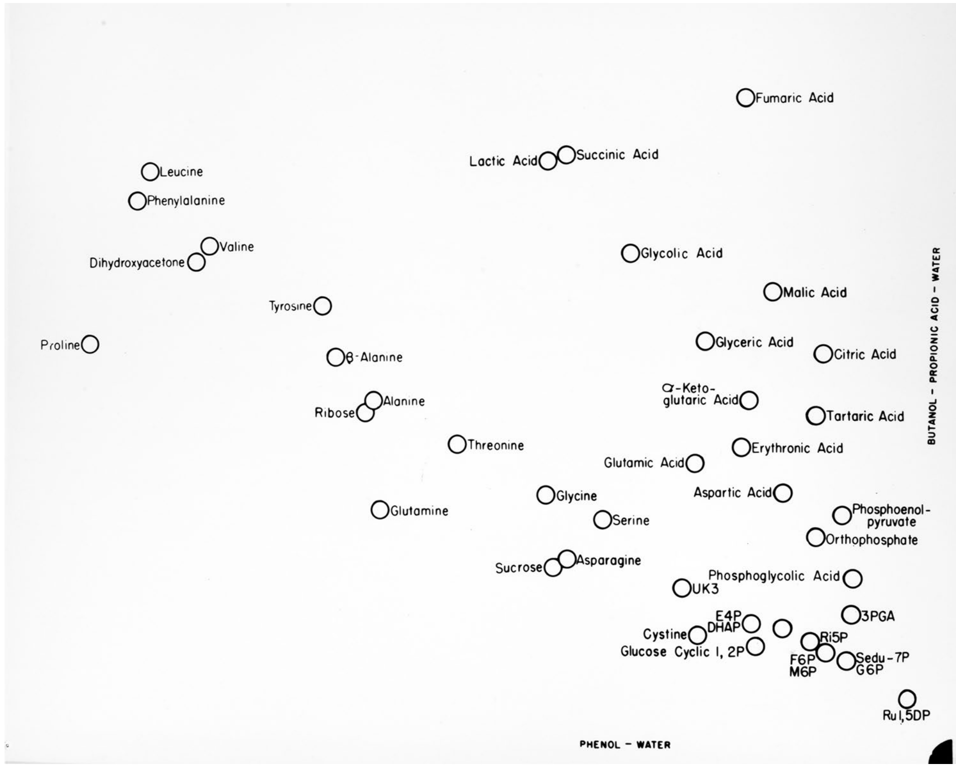

Fig. 5 Benson's keys to autoradiograms. Only after comparing each chromatographic spot with a known standard could the position of each be identified. This took years of highly reproducible precision chemistry, the methods of which were the basis of *Benson's Protocol*. One of his keys mapped the R_f for each of the metabolites using two different mobile phases, Phenol–Water and Butanol–Propionic Acid–Water. Note the position of sedoheptulose and RuDP (RuBP) in the *lower right corner*. Andy focused on the group of compounds in this *corner* because, since 1949, he had discovered that they appeared in the first few seconds of exposure to $^{14}CO_2$ (Benson et al. 1950)

we now probe into Andy's scientific adventures, we show, in Fig. 7, Andy riding astride a crop foliar spray tractor: he clearly enjoyed his scientific and personal endeavors immensely (see Benson 1981).

Andy had the gift of scientific inquiry that he utilized to dig out the truth, recognizing unexpected results; and, trailblazer that he was, he would effectively show his colleagues how to get to the correct destination. A partial listing of Andy's exemplary scientific investigations is presented in Tables 1, 2, 3 and 4, featuring the global scope of his bold explorations. His radioisotopes, listed in Table 1, are grouped according to the elements used and his experiments undertaken across a wide variety of live samples. Early in his research, Andy combined different radioisotopes, such as $^{14}CO_2$ and ^{32}P-phosphate (Benson et al. 1950); later, in the 1960s, Andy combined $^{35}SO_4$ and $^{14}CO_2$.

Of mice and chemists

In the 1930s, Andy worked with Sam Ruben and Martin Kamen using ^{11}C applied to rat lungs. He furthermore utilized this first, but short-lived, radioisotope of carbon in photosynthesis, after the publication by Ruben et al. (1939). With the advent of ^{14}C, Andy synthesized the world's supply of concentrated $^{14}CO_2$ to be used for its fixation in living organisms. When Melvin Calvin invited Andy, in 1946, to set up the laboratory at University of California Berkeley, to find the path of carbon in photosynthesis, Andy began to apply "his" $^{14}CO_2$ to photoautotrophic microbes and algae, listed in Table 2. Isotopes were selectively used based on the goal of the specific experiments. For this, different unicellular, colonial, and thalloid algae were treated with inorganic radioisotopes (see Benson and Bassham 1948; Calvin and Benson 1948; Benson and Calvin 1950; Calvin et al.

Fig. 6 Benson (*left*) with Arthur Nonomura (*right*; one of the authors) monitoring field modulation of glycoregulation in roses; October 1992, Woolf Farms, Waddell, Arizona, USA. Benson enjoyed the intense fragrance of rose blossoms covering thousands of acres in the Valley of the Sun. From the private collection of Dee Benson

Fig. 7 Benson enthusiastically participated in a demonstration of a row crop spray rig; October 1992, Litchfield Park, Arizona. From the private collection of Dee Benson

1951; also see Fig. 4b; Fig S5 in the Supplementary Material; Online Appendix; Table 2).

Membrane chemistry

Higher plants were fed primarily with $^{14}CO_2$ (see Table 3). Using these plants, Andy discovered phosphatidylglycerol and sulfoquinovosyldiglyceride, and proved the importance of membrane phospholipids and sulfolipids in their function. Wada and Murata (2010) dedicated their book on "Lipids" to Benson for his discoveries in this area. Further, Andy studied membrane chemistry through the application of ^{32}P and of ^{35}S in many plants, listed in Table 3.

Benson's biorefineries

Animals were tracked with radiolabeled organic products, such as alcohols, sugars, lipids, and organic acids, since these organisms were capable of metabolizing such compounds; for example, ^{14}C-glucose. Andy obtained complex organic compounds by culturing algae in $^{14}CO_2$ and isolating their specific radiolabeled metabolites; see, for example, ^{14}C-*Dunaliella* (Cooney and Benson 1980). He looked into complex organisms such as zooxanthellae, symbionts in corals and clams (see, e.g., Benson 1990; Tables 2, 4). At that point Andy could have brought the investigation of zooxanthellae to completion, but he did not stop there, for he could now utilize these symbionts as bio-refineries to feed their animal hosts with radiolabeled products that he had isolated from the zooxanthellae. He investigated heterotrophs, such as bacteria and fungi listed in Table 4, and he studied animals including copepods, fishes, rats, hamsters, sheep, and cows (see, e.g., Strickland and Benson 1960; Patton and Benson 1966, 1975; Patton et al. 1977). Andy unveiled aging processes by applying ^{125}I-calcitonin to porcine lung tissue (Fouchereau-Peron et al. 1981) and $^{32}P + ^{45}Ca$ to *Oncorhynchus gorbuscha*, pink salmon (Milhaud et al. 1977), as listed in Table 4.

Table 1 Benson's isotopes

Isotope	Reference
^{74}As	Katayama et al. (1990)
^{80}Br	Strickland and Benson (1960)
^{82}Br	Steim and Benson (1964)
^{60}Co	Strickland and Benson (1960)
^{11}C	Benson (2011)
^{13}C	Gout et al. (2000)
^{14}C	Calvin and Benson (1948), Benson et al. (1950, 1952), Benson (1951), Nordal and Benson (1969)
^{125}I	Fouchereau-Peron et al. (1981)
^{31}P	Strickland and Benson (1960), Steim and Benson (1964)
^{32}P	Strickland and Benson (1960)
$^{32}P + ^{14}C$	Benson et al. (1950), Ferrari and Benson (1961)
$^{32}P + ^{45}Ca$	Milhaud et al. (1977)
^{32}S	Strickland and Benson (1960)
^{35}S	Shibuya et al. (1963, 1965), Miyachi et al. (1966), Busby and Benson (1973)
$^{35}S + ^{14}C$	Benson et al. (1960), Yagi and Benson (1962)
$^{35}S + ^{75}Se$	Nissen and Benson (1964)

Among the 15 different isotopes that Benson employed in his experiments with biological specimens, he utilized ^{11}C, ^{12}C, ^{13}C, ^{14}C, as well as several other isotopes listed

Table 2 Benson's microbes and algae

Organism	Reference; for figures, see Supplementary Material and Online Appendix
Rhodospirillum rubrum	Calvin et al. (1951), Benson (1968), Brooks and Benson (1972); Fig. S22
Chlorella ellipsoidea	Calvin and Benson (1948); Figs. S4 and S10
Chlorella pyrenoidosa	Benson (1987)
Scenedesmus obliquus Gaffron Strain D-23	Calvin and Benson (1948), Benson and Maruo (1958); Figs. S1, S2, S3, S7, S8 and S9
Botryococcus braunii Showa	Nonomura and Benson (1992)
Chaetoceros concavicornis	Figure S18
Cylindrotheca fusiformis	Katayama et al. (1990)
Dunaliella tertiolecta	Cooney and Benson (1980)
Ochromonas danica	Miyachi et al. (1966)
Iridaea laminarioides	Figure S5
Symbiodinium of *H. hippopus*	Figure S21
Euglena gracilis	Figure S6

Table 3 Benson's plants (left column) with references (right column)

Lemna perpusilla (duckweed)	Shibuya et al. (1965)
Aegialitis annulata (club mangrove)	Figure S11
Hordeum vulgare (barley)	Benson and Calvin (1950), Benson (1951); Fig. S13
Geranium sp. (geranium)	Benson (1951)
Glycine max (soybean)	Benson et al. (1952)
Fragaria ananassa (strawberry)	Steim and Benson (1964)
Nicotiana tabacum (tobacco)	Figure S14
Tetragonia expansa (Botany Bay spinach)	Figure S15
Zea mays (corn)	Nissen and Benson (1964)
Helianthus annuus (sunflower) *Triticum aestivum* (wheat) *Zostera marina* (eelgrass) *Phyllospadix torreyi* (surfgrass)	Figure S24
Euphorbia resinifera (resin spurge)	Nordal and Benson (1969)
Medicago sativa (alfafa) *Erythrina crista-galli* (coral tree)	Lee and Benson (1972)
Acer pseudoplatanus (sycamore)	Gout et al. (2000)
Beta vulgaris (sugar beet)	Biel et al. (2010)
Beta vulgaris ssp. *cicla* (Swiss chard) *Raphanus sativus* (radish)	Nonomura and Benson (2014)

Chemist and biologist

Since Andy was a chemist, partnership with biologists was critical for determining suitable species to explore (Buchanan et al. 2007; Lichtenthaler et al. 2008; Buchanan and Douce 2015; Nonomura et al. 2016). We note that our present historical article on Andy is indeed presented from a biological perspective. In order to define the path of sulfocarbohydrates, Andy investigated the coral tree because of its alkaloids and alfalfa for its accumulation of sulfolactates (Table 3; Lee and Benson 1972). For Andy's step up to mixotrophy, he worked on *Euglena* (see Table 2; Figure S6). Foraminifera are amoeboid heterotrophs and some species may contain algal symbionts, while others are kleptoplastic (where chloroplasts are sequestered by the host organism), and Andy applied therefore autoradiography to *Foraminifera vertebralis* (see Table 4; Figure S19 in the Supplementary Material; also see Online Appendix).

By circumnavigating the seas to apply ^{74}As to gossamer diatoms (Katayama et al. 1990; Busby and Benson 1973), clams (Benson 1990), lobster (Cooney and Benson 1980), and zooxanthellae, Andy found how arsenic is regulated in nature and the relationship of Si to S-metabolism (see Tables 1, 2, 4).

As is well known to most of us, Andy worked to define the carbon reactions of photosynthesis beginning in 1947 (Aronoff et al. 1947) to the end of 1955 (see, e.g., Lichtenthaler et al. 2015; Nonomura et al. 2016). Thereafter, Andy was also involved in studies on cell and organelle membranes (Benson 1966), and in particular chloroplast membranes (Douce et al. 1973). Plants used by Andy spanned a spectrum of diversity including barley (Benson and Calvin 1950; Benson 1951), soybean (Benson et al. 1952), *Euphorbia* (Nordal and Benson 1969), strawberry (Steim and Benson 1964), *Lemna perpusilla* (Shibuya et al. 1965), Botany Bay spinach, mangrove, tobacco, and wheat. Plants listed in Table 3 were the stars of his experiments.

As early as 1949, Andy had investigated the contribution of the simplest organic compound, methanol, because he had observed that ^{14}C-methanol (^{14}C-MeOH) was metabolized as rapidly as $^{14}CO_2$ in *Chlorella* and *Scenedesmus* (Calvin and Benson 1949). This interest in methanol had been ongoing: one of us (Robert Cooney, personal communication, 2017) had applied, in 1978, ~0.3% ^{14}C-MeOH to cultures of *Chlorella ellipsoidea*, resulting in a single chromatographic spot (Fig. 8a); further, pretreatment of *Chlorella ellipsoidea* with low concentrations of MeOH was followed by $^{14}CO_2$ treatment

Table 4 Benson's heterotrophs

Flavobacterium sp. (soil bacteria)	Martelli and Benson (1964)
Saccharomyces sp. (yeast)	Benson et al. (1950)
Aspergillus niger (mold)	Nissen and Benson (1964)
Foraminifera vertebralis	Figure S19
Pocillopora capitata	Patton et al. (1977)
Pocillopora damicornis *Pectinia lactuca* *Sarcophyton trocheliophorum* *Acropora formosa* (corals)	Benson and Muscatine (1974) Figure S20
Acanthaster ellisii (Crown of Thorns, sea star)	Benson et al. (1975)
Tridacna maxima (small giant clam)	Benson (1990)
Homarus americanus (lobster)	Cooney and Benson (1980)
Oncorhynchus gorbuscha (pink salmon)	Milhaud et al. (1977)
Engraulis mordax (northern anchovy) *Trachurus symmetricus* (jack mackerel) *Scomber japonicus* (Pacific mackerel) *Morone saxatilis* (striped bass)	Patton et al. (1975)
Calanus helgolandicus (calanoid copepod)	Holtz et al. (1973)
Ovis aries (sheep)	Benson (1987)
Rattus norvegicus (rat) *Bos* sp. (beef) *Ovis aries* (sheep)	Strickland and Benson (1960) Figure S16
Mesocricetus auratus (golden hamster) *Rattus norvegicus* (albino rat)	Strickland and Benson (1961) Figure S23
Bovinae (bovine)	Benson (1987), Flipse and Benson (1957)
Bos taurus (Holstein cow)	Patton and Benson (1966)
Sus scrofa domesticus (pig)	Fouchereau-Peron et al. (1981)

(Fig. 8b). Benson's group resumed studies on plant responses to MeOH in 1992, when one of us (Nonomura) demonstrated visibly discernible reduction of midday wilt in fields of roses, cotton, and cabbage treated with 20–50% MeOH in modified Hoagland solution (for details, see: Nonomura and Benson 1992; Benson et al. 1992). This team of a chemist (Benson) and a botanist (Nonomura) followed through with experiments on Swiss Chard and barley leaves fed with $^{14}CO_2$ and pretreated with 20% MeOH. When this experiment was followed by exposure of leaves to saturated O_2, results showed unexpected inhibition of glycolic acid compared to that in controls without MeOH. In dozens of replicates, a dark glycolic acid (Schou et al. 1950) spot was clearly labeled in the control autoradiogram (Fig. 8c). In contrast, the MeOH autoradiogram (Fig. 8d) showed a faint gray trace in that location. Benson and Nonomura then joined with Roland Douce's group in Grenoble, France, in tracking MeOH to methyl glucoside (MeG; Gout et al. 2000); and thereafter, observed plant and algal growth responses with Valrie Gerard (Benson et al. 2009). A decade later, sugar beets were treated with ^{14}C-MeG (see Biel et al. 2010); after 22 h, autoradiography of whole sugar beet plants showed a single spot of ninhydrin-N-linked-^{14}C-MeG (Fig. 9), while the untreated control showed nothing in that position. As captured in these autoradiograms, Andy's pursuit of ^{14}C-MeOH spanning 65 years led to new ideas on modulation of glycoregulation.

We believe that Andy's greatest virtue was his perseverance, and we speculate that this may have been partly responsible for his longevity. Andy brought the finest details of life to the fore through development of isotopic applications (Benson 2011). He even investigated mushrooms (Vaskovsky et al. 1991), but why be limited to a crustacean, fish, or fungus when one can chart an entire ecosystem? After all, ecosystems are defined in part by the transfer of energy from one species to the next. Thus, the transfer of energy, for example, from corals to fishes (Benson and Muscatine 1974) or through wax from copepods, was charted by Andy and one of us (see, e.g., Holtz et al. 1973; Table 4) via radiolabels in the food chain.

Greatest benefit

When Andy needed a new technology, he had the vision to design and construct it from the ground up and then improve upon it. In the early years, Andy worked closely with James

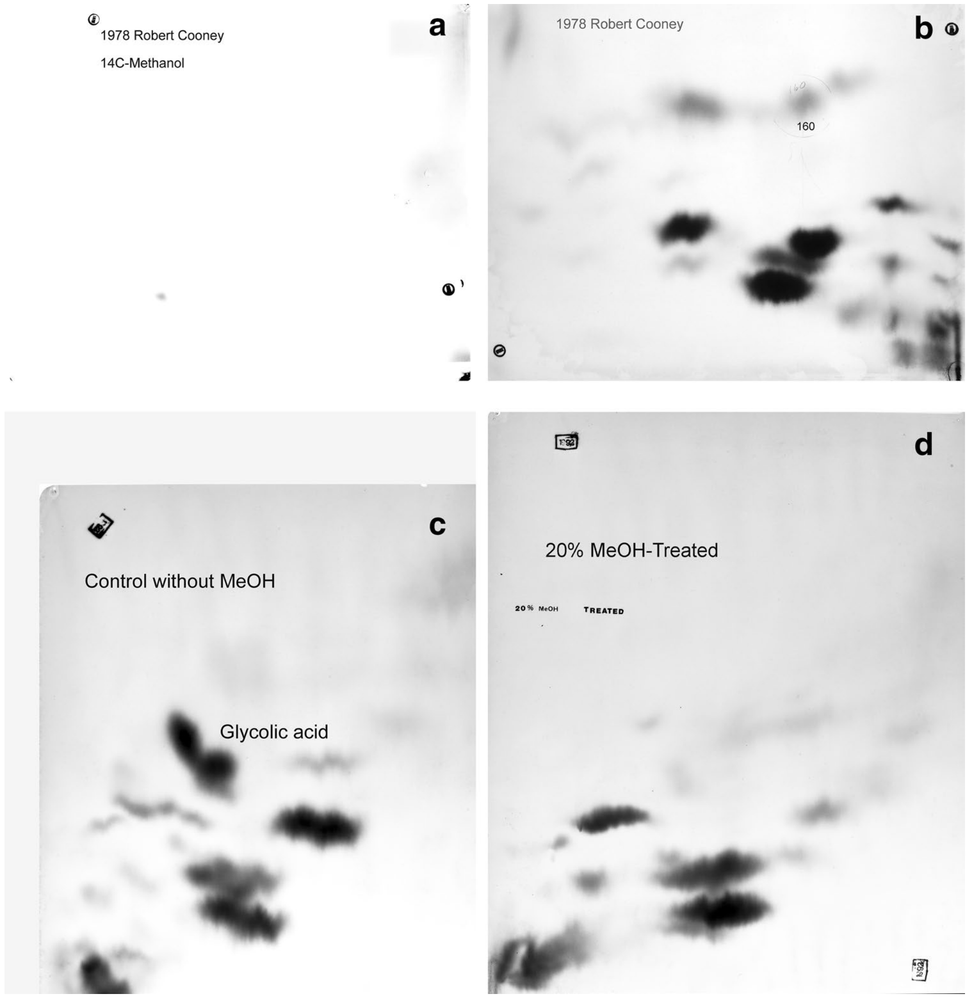

Fig. 8 **a** Autoradiograms of *Chlorella ellipsoidea* fed with ^{14}C-MeOH (a 1978 experiment); **b** treatment with ~0.3% MeOH was followed by $^{14}CO_2$ autoradiograms from Robert Cooney; **c** photorespiration in Swiss chard leaf fed with $^{14}CO_2$ revealed glycolic acid in controls in O_2, without MeOH; **d** pretreated with 20% MeOH; leaves in O_2 did not show glycolic acid (see Benson and Nonomura 1992 for the disclosure of these results, but in which these autoradiograms were not published)

A. Bassham in the Old Radiation Laboratory (Benson 2002; Bassham 2003; Govindjee et al. 2016; Dent et al. 1947). As example of their pioneering work, immediately after finding organic acid products of $^{14}CO_2$, they (Benson and Bassham 1948) developed sophisticated chemical synthesis and biological methods to biosynthesize, isolate, and apply ^{14}C-succinic and ^{14}C-malic acids in their investigations. Within months, *The Path of Carbon in Photosynthesis* (Calvin and Benson 1948) was published. This profound innovative approach was repeatedly demonstrated, as in the case of his neutron activation studies (Benson 1987). As soon as a new technology became available, Andy made fresh discoveries in other areas with a multitude of colleagues. For example, refraction of light through glass microbeads was found to enhance its intensity at the phylloplane (Nonomura and Benson 2012). Investigations of in vivo ^{13}C nuclear magnetic resonance kept Andy on the cutting edge of science with his past doctoral associates and colleagues (Gout et al. 2000), and this too when he was 83-years old. With one of us (Nonomura), the first recognition of a specific glycoconjugate pathway was translated into the field of modulation of glycoregulation (Nonomura and Benson 2014). We are thrilled to note that, at age 97, Andy was recognized by Brandt iHammer for "*Conferring the Greatest Benefit on Mankind*" (Buchanan and Douce 2015).

When Andy applied each of the different radioisotopes to plants or animals, he discovered *The Path of Sulfocarbohydrates, The Path of Sulfolipids, The Path of Phospholipids, The Path of Arsinolipids, The Path via Neutron Activation, The Path of Photosynthates in Animals, The Path to Waxes, The Path from Waxes into the Marine Food Chain, The Path to Membranes*, and *The Path to Glycoregulation*. Each stepping stone laid down by Andy paved the path to discoveries of higher and higher order.

We note that Andy's legacy was never confined to photosynthesis since he had applied his protocol to mixotrophs, heterotrophs, and ecosystems. In fact, his earliest investigations with ^{11}C-phosgene were on rats, while some of his later studies were on marine ecosystems. During his lifetime, Andy demonstrated wide-ranging application of his philosophy of isotopic tracers, while others followed him.

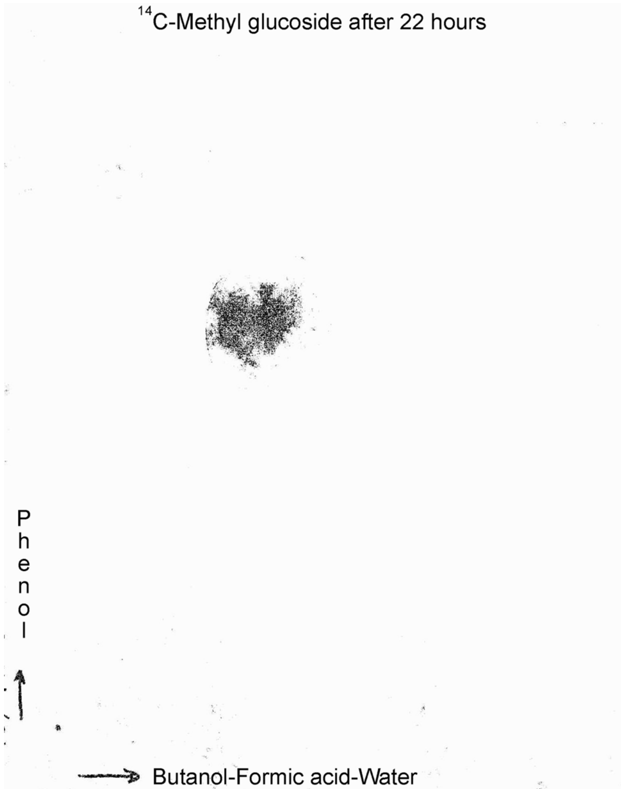

Fig. 9 Autoradiography of *Beta vulgaris* (sugar beet) showed a single spot of ninhydrin-N-linked-^{14}C-MeG (see Biel et al. 2010 in which the results were disclosed, but the 2D-paper chromatogram was not published)

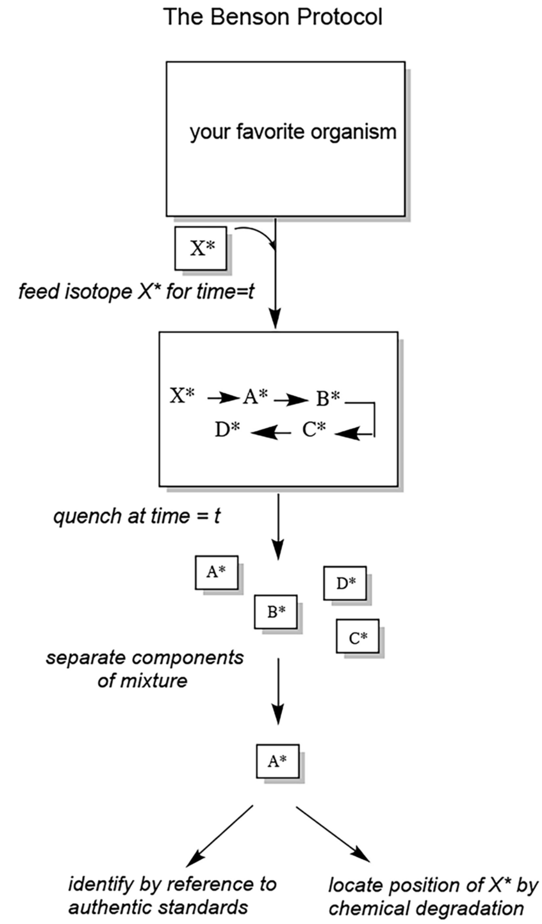

Fig. 10 Flow chart of Benson's Protocol

Benson's protocol, then and now

Following World War II (1939–1945), the stage was set for attempts to reveal the hitherto mysterious processes of metabolism. A radioactive form of carbon with a suitably long half-life had been discovered (see Kamen 1963) and the technique of 2D-paper chromatography had been developed for resolving complex mixtures of closely related compounds (Martin and Synge 1941). Andy was the first to apply these methods to a complex metabolic system, the path of carbon in photosynthesis. In its generic form, Benson's Protocol (Fig. 10) is quite straightforward, although demanding in technical skill and considerable chemical know-how.

A particularly novel application of Benson's Protocol that took advantage of Andy's extensive knowledge of the path of carbon in photosynthesis is exemplified by his novel discovery of the mechanism through which the herbicide methane arsonate acts in the eradication of Johnson grass (*Sorghum halepense*) near sugar cane fields (Knowles and Benson 1983). Methane arsonate is toxic to the C4 photosynthetic pathway employed by Johnson grass, yet it does not affect C4 Bermuda grass. Investigation of the pattern of photosynthesis by autoradiography revealed accumulation of malate and significant reduction of sucrose in methane arsonate-treated leaves. This was attributed to an inhibition of NADP^{+}-malic enzyme. Their studies suggested that oxidation of key sulfhydryl groups by methane arsonate inhibited CO_2 release from malate in bundle sheath cells, depriving the plant of its source of carbon for sucrose production. In the case of Bermuda grass, differential mechanisms of carbon transport to the bundle sheath cells and high transaminase activity may be responsible for the insensitivity of Bermuda grass to methane arsonate. In the hands of other skilled chemists, complex pathways leading to the synthesis of sterols were determined (see Cornforth 2002). In this manner, *Benson's Protocol* was to form the basis for what might be considered the golden age of metabolic biology.

Science and technology march on; even today, however, the elements of Benson's Protocol can still be recognized. Radioactive isotopes are being replaced with stable isotopes (e.g., ^{13}C, ^{15}N, and ^{18}O) that are now available in highly enriched form. The limited resolution of paper chromatography has been largely replaced by liquid and gas chromatographic methods that have the additional advantage that they can be coupled to mass spectrometry. This allows the more-or-less unequivocal identification of the compounds in question and, from the fragmentation pattern, the quantitative distribution of the isotope. Beyond the ability to interpret a mass spectrum, next-to-no chemical knowhow is needed. Examples of the modern application of Benson's Protocol, fittingly from the field of photosynthesis/photorespiration, with ^{13}C (Gout et al. 2000; Flanagan et al. 2006) and another with ^{18}O (Berry et al. 1978), illustrate the point.

Conceptually, these and other metabolic pathways arose from applying a variant of Benson's Protocol. *Beyond science, Andy was first and foremost our great friend, whether at home, in the laboratory, or abroad* (see tributes from Govindjee 2010; Biel and Fomina 2015; Nonomura et al. 2016; Buchanan et al. 2016 for examples). Fondly remembered camaraderie from one of us (Barry Holtz) is shown in Fig. 11, and another (Govindjee) remembers the fun time[1] he had with Andy when he was driven with his wife Rajni by Andy in his old BMW (Bavarian Motor Works automobile; see Fig. 12a). Figure 12b shows another memorable occasion in 2001 when Andy and Govindjee each gave their own historical lecture on "carbon fixation" and "water oxidation and NADP reduction" aspects, respectively, of oxygenic photosynthesis (see Govindjee 2010).

Concluding remarks

No phase of life's unfolding story is more brilliant with the fire of heroic purpose than the crossing of these radioisotope tracers with the spectrum of life (Buchanan et al. 2016). Even the 1961 Nobel Prize to Melvin Calvin, in chemistry, was trifling beside these volumes of enlightenment of the interactions of air, minerals, water, light, and the food chains of life. And it was Benson (1981) who explored unknown metabolic worlds and adventurously persevered until he found answers to the most significant questions.

[1] As soon as the Govindjee's opened the car door, they discovered that there were no seats in the back of Andy's car. Rajni asked: "Where should I sit?" Andy replied: "On the nice seat made up by all the old telephone directories". So, she did. When Andy started driving, Govindjee asked Andy: "Where are the seat belts?" Andy replied: "Well, well, there are no seat belts." Govindjee said: "Wouldn't the police give you a ticket?" He responded: "No, not really; this is a very old car and it didn't come with seat belts and it is almost impossible to put seat belts here". So, we went on the drive out, all of us laughing.

Fig. 11 A 1976 photograph of Benson (*left*) with Barry Holtz (one of the authors). Benson is wearing one of his signature bowties and a broad smile; they were at a conference. From the private collection of Barry Holtz

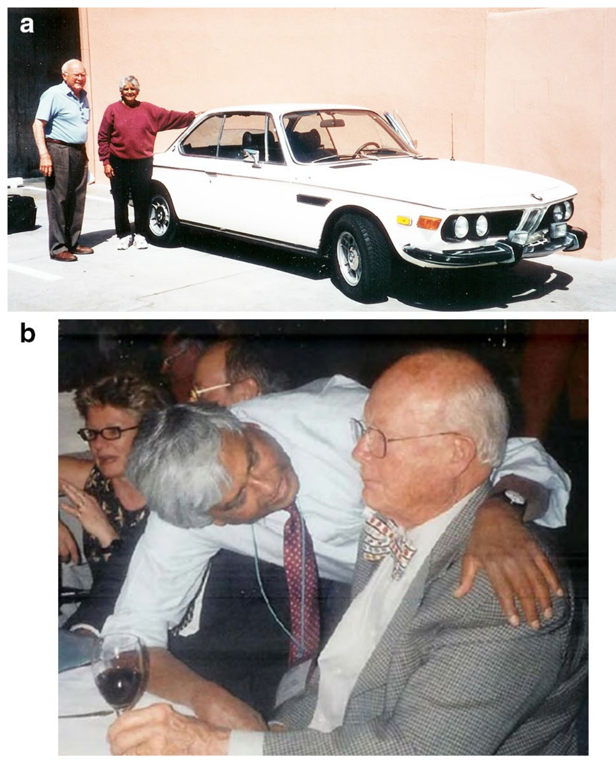

Fig. 12 **a** A 2001 photograph of Andy Benson and Rajni Govindjee with Benson's vintage BMW before Andy gave Govindjee and Rajni a ride (see footnote 1 for a story). From the private collection of Govindjee; **b** A 2001 photograph of Benson, wearing a bowtie, and one of the authors (Govindjee) at a dinner in Brisbane, Australia. (Fig. 3 from Govindjee 2010, reproduced with Springer's permission)

We end this Tribute by showing photographs of Andy with a few other scientists. Figure 13a shows him with Ralph Lewin, the two having had a hand in designing

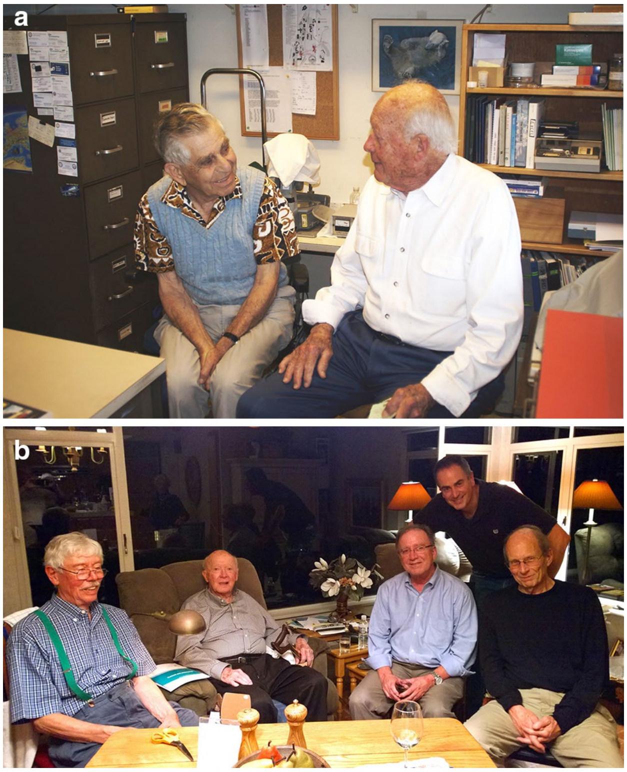

Fig. 13 **a** A 2007 photograph of Ralph A. Lewin (*left*) and Benson (*right*) discussing matters of common interest (algae in hairs of captive polar bears, plant physiology, and discoveries); **b** A 2014 photograph at Benson's residence in La Jolla, California, USA. From *left to right* Roland Douce, Andrew A. Benson, Barry Holtz, Julian Schroeder, and Bob B. Buchanan (see Buchanan and Douce 2015). From the private collection of Arthur Nonomura

Fig. 14 Never one to be lab-bound, Benson met one of us, George Lorimer, in San Diego, and trekked inland to agricultural fields in Arizona during a sweltering 45 °C day in 1990. Benson (*left*) and Nonomura (*right*) standing in an irrigated farm field (see footnote 2 for a story). Nonomura handed his camera over to Lorimer prior to crossing the irrigation ditch onto the field with Benson; photograph by George Lorimer

Fig. 15 A 2007 photograph of Andrew Alm Benson standing before the blackboard in his office, holding a copy of the Proceedings of the National Academy of Sciences of USA, which he had read cover to cover. From the private collection of Arthur Nonomura

and "building" Hubbs Hall where they did their own research; Fig. 13b shows Andy with others including Barry Holtz (coauthor of this Tribute), Roland Douce, and Bob Buchanan who have written papers honoring Andy (see, e.g., Buchanan and Douce 2015 and a 2012 conversation with Buchanan at: https://www.youtube.com/watch?v=GfQQJ2vR_xE); Fig. 14 shows Andy actively involved in field research with Arthur[2]; and Fig. 15 shows a fitting photograph to end Andy's centenary celebration—it

[2] Andy traveled to the ends of the Earth to undertake research in the field—see for example Nonomura et al. (2016); particularly on a cruise in the Indian Ocean to the Republic of Seychelles with Karl Biel (one of us), while witnessing the green flash in Hawaii with Kay and Robert Cooney (one of us), and by adventurous investigations at Alert Bay, BC, Canada with Andy's wife (Dee Benson) and Barry Holtz (one of us). Regarding Fig. 14, in preparation for a particularly active visit to inspect replicated field trials in the desert of Arizona, one of us (Arthur Nonomura) advised Andy to wear comfortable shoes with rubber soles for crossing ditches. Seeing that Andy was wearing white shoes and that George Lorimer (one of us) and Andy Benson were wearing suits, Arthur "dressed up" too by donning his own white shoes. They visited hundreds of acres of cotton, sighting midday wilt of controls in contrast to turgid plants in treated sections. In order to inspect the moisture of the farm's sandy loam soils, they stopped at the edge of a field that was accessible only by traversing a 1.5 m-wide irrigation ditch. This required a specific choreography to step across the concrete mouth without falling into the watery ditch. Andy, belying his 73 years, nimbly stepped across the ditch, reminding us that he was born and raised on a large farm community in Central California.

shows Andy in front of a black board where he had just written the chemical structure of a compound (a frequent *happenstance*, when he talked with others about his or their research). We all miss you, Andy.

Acknowledgements The authors express their sincere gratitude to Dee Benson and Victor Vacquier. The field pictures (Figs. 6 and 7) were provided by Dee Benson, who was by Andy's side during their 45 years of marriage as they traveled on scientific excursions and life adventures. Dee and Andy had great fun! We give special thanks to Thomas D. Sharkey, William W. Adams III, Hartmut Lichtenthaler, an anonymous reviewer, and Rajni Govindjee for their help during the preparation of this manuscript.

References

Aronoff S, Benson AA, Hassid WZ, Calvin M (1947) Distribution of C14 in photosynthesizing barley seedlings. Science 105:664–665

Bassham JA (2003) Mapping the carbon reduction cycle: a personal retrospective. Photosynth Res 76:35–52

Benson AA (1951) The sequence of formation of hexoses during photosynthesis. Arch Biochem Biophys 32:223–224

Benson AA (1966) On the orientation of lipids in chloroplast and cell membranes. J Am Oil Chem Soc 43:265–270

Benson AA (1968) Neutron activation. Kagaku no Ryoiki (Chemistry and Related Sciences; Japanese English) 22(1):03–104

Benson AA (1981) Molecular exploration in unknown metabolic worlds. In: Kagayama M, Nakamura K, Oshima T, Uchida T (eds) Science and scientists. Japan Scientific Societies Press, Tokyo, pp 207–212

Benson AA (1987) Radiochromatographic exploration. J Am Oil Chem Soc 64:1309–1314

Benson AA (1990) Arsenic deputation via the Tridacna gill membrane. Z Naturforsch C 45:793–796

Benson AA (2002) Following the path of carbon in photosynthesis: a personal story. Photosynth Res 73:29–49

Benson AA (2011) Adventures with carbons 11, 12, 13 and 14. Photosynth Res 110:9–12

Benson AA, Bassham JA (1948) Chemical degradation of isotopic succinic and malic acids. J Am Chem Soc 70:3939–3940

Benson AA, Calvin M (1950) The path of carbon in photosynthesis VII. Respiration and photosynthesis. J Exp Bot 1:63–68

Benson AA, Maruo B (1958) Radiochemical identification of diglycerophosphate and its probable role in lipid synthesis by plants. Radioisotopes in scientific research, vol IV. In: Proceedings of the 1st UNESCO International Conference, Paris 1957, Pergamon Press, London

Benson AA, Muscatine L (1974) Wax in coral mucus: energy transfer from corals to reef fishes. Limnol Oceanogr 19:810–814

Benson AA, Nonomura AM (1992) The path of carbon in photosynthesis: methanol inhibition of glycolic acid accumulation. In: Murata N (ed) Research in photosynthesis. Proceedings of the 9th International Congress 34, 196, P-522. Kluwer, Dordrecht

Benson AA, Bassham JA, Calvin M, Goodale TC, Haas VA, Stepka W (1950) The path of carbon in photosynthesis. V. Paper chromatography and radioautography of the products. J Am Chem Soc 72:1710–1718

Benson AA, Bassham JA, Calvin M, Hall AG, Hirsch HB, Kawaguchi S, Lynch V, Tolbert NE (1952) The path of carbon in photosynthesis. XV. Ribulose and sedoheptulose. J Biol Chem 196:703–716

Benson AA, Wiser R, Maruo B (1960) The sulfolipids of brain and chloroplasts. In: Popjak G (ed) Proceedings of 4th International Congress Biochemistry, Vienna, 1958, Biochem of Lipids, vol 5, pp 8–10. Pergamon Press, London

Benson AA, Patton JS, Field CE (1975) Wax digestion in a crown-of-thorns starfish. Comp Biochem Physiol 52B:339–340

Benson AA, Stein JL, Nonomura AM (1992) Methanol effect on leaf photorespiration. FASEB J 7(7):1110 (**Abstract #340**)

Benson AA, Nonomura AM, Gerard VA (2009) The path of carbon in photosynthesis. XXV. Plant and algal growth responses to glycopyranosides. J Plant Nutr 32(7):1185–1200. http://www.tandfonline.com/doi/pdf/10.1080/01904160902943205

Berry JA, Osmond CB, Lorimer GH (1978) Fixation of $^{18}O_2$ during photorespiration. Plant Physiol 62:954–967

Biel KY, Fomina IR (2015) Benson–Bassham–Calvin cycle contribution to the organic life on our planet. Photosynthetica 53:161–167

Biel KY, Nonomura AM, Benson AA, Nishio JN (2010) The path of carbon in photosynthesis. XXVI. Uptake and transport of methylglucopyranoside throughout plants. J Plant Nutr 33(6):902–913. http://www.tandfonline.com/doi/pdf/10.1080/01904161003669897

Brooks JL, Benson AA (1972) Studies on the structure of an ornithine-containing lipid from *Rhodospirillum rubrum*. Arch Biochem Biophys 152:347–355

Buchanan BB, Douce R (2015) Andrew Benson honored on birthday no. 97. Photosynth Res 123:115–116

Buchanan JG, Bassham JA, Benson AA, Bradley DF, Calvin M, Daus LL, Goodman M, Hayes PM, Lynch VH, Norris LT, Wilson AT (1952) The path of carbon in photosynthesis. XVII. Phosphorus compounds as intermediates in photosynthesis. In: McElroy WD, Glass B (eds) Phosphorus metabolism, vol II. The Johns Hopkins Press, Baltimore, pp 440–459

Buchanan BB, Douce R, Lichtenthaler HK (2007) Andrew A. Benson. Photosynth Res 92:143–144

Buchanan BB, Douce R, Govindjee, Lichtenthaler HK (2016) Andrew A. Benson 1917–2015. Biographical Memoirs of the National Academy of Sciences, USA, pp 1–16, http://www.NASonline.org/memoirs

Busby WF, Benson AA (1973) Sulfonic acid metabolism in the diatom *Navicula pelliculosa*. Plant Cell Physiol 14:1123–1132

Calvin M, Benson AA (1948) The path of carbon in photosynthesis. Science 107:476–480

Calvin M, Benson AA (1949) The path of carbon in photosynthesis IV: the identity and sequence of intermediates in sucrose synthesis. Science 109:140–142

Calvin M, Bassham JA, Benson AA, Lynch V H, Ouellet C, Schou L, Stepka W, Tolbert N E (1951) The path of carbon in photosynthesis. X. Carbon dioxide assimilation in plants. In: Danielli JF, Brown R (eds) Carbon dioxide fixation and photosynthesis. Proceedings of the Society for Experimental Biology (Great Britain) vol 5, pp 284–305

Cooney RV, Benson AA (1980) Arsenic metabolism in *Homarus americanus*. Chemosphere 9:335–341

Cornforth JW (2002) Sterol biosynthesis: the early days. Biochem Biophys Res Commun 292:1129–1138

Dent CE, Stepka W, Steward FC (1947) Detection of the free amino acids of plant cells by partition chromatography. Nature 160:682–683

DOE (1962) The Ernest Orlando Lawrence Award, Laureate Andrew A. Benson, 1962. Life sciences: for outstanding contributions to elucidation of the carbon reduction cycle in photosynthesis through his development of double labeling techniques employing C14, H3, and P32. Andrew A. Benson, 1962 | U.S. DOE Office of Science (SC).pdf, Office of Science, U.S. Department of Energy, 1000 Independence Ave.,

SW, Washington, DC 20585 USA, https://science.energy.gov/lawrence/award-laureates/1960s/benson/
Douce R, Holtz RB, Benson AA (1973) Isolation and properties of the envelope of spinach chloroplasts. J Biol Chem 248:7215–7222
Ferrari RA, Benson AA (1961) The path of carbon in photosynthesis of the lipids. Arch Biochem Biophys 93:185–192
Flanagan IL, MacLeod JK, Williams JA (2006) A re-investigation of the path of carbon in photosynthesis utilizing GC/MS methodology. Unequivocal verification of the participation of octulose phosphates in the pathway. Photosynth Res 90:149–159
Flipse RJ, Benson AA (1957) Catabolism of glycine-C14 by washed bovine spermatozoa. Exp Cell Res 13:611–614
Fomina IR, Biel KY (2016) Photosynthetic carbon metabolism: strategy of adaptation over evolutionary history. In: Allakhverdiev SI (ed) Photosynthesis: new approaches to the molecular, cellular, and organismal levels. Scrivener Publishing, LLC, Austin, pp 233–326
Fouchereau-Peron M, Mukhtar MS, Benson AA, Milhaud G (1981) Characterization of specific receptors for calcitonin in porcine lung. Proc Natl Acad Sci USA 78(6):3973–3975
Gout E, Aubert S, Bligny R, Rebeille F, Nonomura A, Benson AA, Douce R (2000) Metabolism of methanol in plant cells. Carbon-13 nuclear magnetic resonance studies in Sycamore. Plant Physiol 123:287–296
Govindjee (2010) Celebrating Andrew Alm Benson's 93rd birthday. Photosynth Res 105:201–208
Govindjee, Bassham H, Bassham S (2016) Remembering James Alan Bassham (1922–2012). Photosynth Res 128:3–13
Holtz RB, Marquez ED, Benson AA (1973) Wax ester biosynthesis by isolated membrane fractions from calanoid copepods. Comp Biochem Physiol 45B(3):585–591
Kamen MD (1963) History of carbon-14. Science 141:861–862
Katayama M, Sugawa-Katayama Y, Benson AA (1990) Effect of selenium on arsenic metabolism in *Cylindrotheca fusiformis*. Appl Organomet Chem 4:213–221
Knowles FC, Benson AA (1983) Mode of action of a herbicide: Johnson grass and methanearsonic acid. Plant Physiol 71:235–240
Lee RF, Benson AA (1972) The metabolism of 35-glyceryl sulfoquinovoside by the coral tree, *Erythrina crista-galli* and alfalfa, *Medicago sativa*. Biochim Biophys Acta 261:35–37
Lichtenthaler HK, Buchanan B, Douce R (2008) Honoring A. Benson. Photosynth Res 96:181–184
Lichtenthaler HK, Buchanan BB, Douce R, Govindjee (2015) Andrew A. Benson, 1917–2015. Photosynth Res 124:131–135
Martelli HL, Benson AA (1964) Sulfocarbohydrate metabolism I. Bacterial production and utilization of sulfoacetate. Biochim Biophys Acta 93(1):169–171
Martin AJP, Synge RLM (1941) A new form of chromatogram employing two liquid phases. Biochem J 35:1358–1368
Milhaud G, Rankin JC, Bolis L, Benson AA (1977) Calcitonin: its hormonal action on the gill. Proc Natl Acad Sci USA 74:4693–4696
Miyachi S, Miyachi S, Benson AA (1966) Sulfolipid in *Ochromonas danica*. J Protozool 13(7):6–78
Nissen P, Benson AA (1964) Absence of selenate esters and "selenolipid" in plants. Biochim Biophys Acta 82:400–402
Nonomura AM, Benson AA (1992) The path of carbon in photosynthesis: improved crop yields with methanol. Proc Natl Acad Sci USA 89:9794–9798 (**Number XXIV in the series**)
Nonomura AM, Benson AA (2012) The path of carbon in photosynthesis. XXIX. Glass microbeads. J Plant Nutr 35:1896–1909
Nonomura A, Benson AA (2013) The path of carbon in photosynthesis. XXX. α-Mannosides. In: Dubinsky Z (ed) Photosynthesis in tech, Janeza Trdine 9, 51000 Rijeka, Croatia, pp 3–22. ISBN: 978-953-51-1161-0 Link to this article: http://www.intechopen.com/books/photosynthesis/the-path-of-carbon-in-photosynthesis-xxx-mannosides
Nonomura AM, Benson AA (2014) The path of carbon in photosynthesis. XXXI. The role of lectins. J Plant Nutr 37:785–794
Nonomura AM, Lorimer G, Holtz RB, Vacquier V, Biel KY, Govindjee (2016) Andrew A. Benson: personal recollections. Photosynth Res 127:369–378
Nordal A, Benson AA (1969) Phorbic acid biosynthesis in the latex vessel system of *Euphorbia*. Plant Physiol 44:78–84
Patton JS, Benson AA (1966) Phytol metabolism in the bovine. Biochim Biophys Acta 125:22–32
Patton JS, Benson AA (1975) A comparative study of wax ester digestion in fish. Comp Biochem Physiol 52B:111–116
Patton JS, Nevenzel JC, Benson AA (1975) Specificity of digestive lipases in hydrolysis of wax esters and triglycerides studied in anchovy and other selected fish. Lipids 10:575–583
Patton JS, Abraham S, Benson AA (1977) Lipogenesis in the intact coral *Pocillopora capitata* and its isolated zooxanthellae: evidence for a light-driven carbon cycle between symbiont and host. Marine Biol 44:235–247
Ruben S, Kamen MD, Hassid WZ, DeVault DC (1939) Photosynthesis with radio-carbon. Science 90:570–571
Schou L, Benson AA, Bassham JA, Calvin M (1950) The path of carbon in photosynthesis, XI. The role of glycolic acid. Physiol Plant 3:487–495
Shibuya I, Yagi T, Benson AA (1963) Sulfonic acids in algae. In: Microalgae and photosynthetic bacteria. Japanese society of plant physiologists. University of Tokyo Press, Tokyo, pp 627–636
Shibuya I, Maruo B, B enson AA (1965) Sulfolipid localization in lamellar lipoprotein. Plant Physiol 40:1251–1256
Steim JM, Benson AA (1964) Derivative activation chromatography. Anal Biochem 9:21–34
Strickland EH, Benson AA (1960) Neutron activation paper chromatographic analysis of phosphatides in mammalian cell fractions. Arch Biochem Biophys 88:344–348
Strickland EH, Benson AA (1961) Synthesis of ^{14}C glycerol from ^{14}C glucose in the rat and hamster duodenum. Biochim Biophys Acta 52:586–587
Vaskovsky VE, Khotimchcnko SV, Benson AA (1991) Identification of diacylglycero 4′-*O*-(*N, N,N*-triniethyi)hornoserine in mushrooms. Lipids 26:254–256
Wada H, Murata N (eds) (2010) Lipids in photosynthesis. Advances in photosynthesis and respiration including bioenergy and other processs, vol 20. Springer, Dordrecht
Yagi T, Benson AA (1962) Plant sulfolipid. V. Lysosulfolipid formation: acids in algae. Biochim Biophys Acta 57:601–603