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# THE $C_2$ OXIDATIVE PHOTOSYNTHETIC CARBON CYCLE

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ABSTRACT

The $C_2$ oxidative photosynthetic carbon cycle plus the $C_3$ reductive photosynthetic carbon cycle coexist. Both are initiated by Rubisco, use about equal amounts of energy, must regenerate RuBP, and result in exchanges of $CO_2$ and $O_2$ to establish rates of net photosynthesis, $CO_2$ and $O_2$ compensation points, and the ratio of $CO_2$ and $O_2$ in the atmosphere. These concepts evolved from research on $O_2$ inhibition, glycolate metabolism, leaf peroxisomes, photorespiration, $^{18}O_2/^{16}O_2$ exchange, $CO_2$ concentrating processes, and a requirement for the oxygenase activity of Rubisco. Nearly 80 years of research on these topics are unified under the one process of photosynthetic carbon metabolism and its self-regulation.

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## *Introduction*

I chose the subject of the $C_2$ cycle for my prefatory chapter because this part of photosynthetic carbon metabolism needs to be better understood. It represents a part of my research that occurred in bits and pieces over many years. This synthesis is hindsight. For a long time I did not foresee conclusions to be drawn from research on glycolate metabolism. Now the term "$C_2$ oxidative photosynthetic carbon cycle" parallels the nomenclature for the "$C_3$ reductive photosynthetic carbon cycle" (Figure 1). The term "photorespiratory carbon cycle" should not be used for the $C_2$ cycle, because that implies photorespiration is a separate process with separate $CO_2$ and $O_2$ pools. Rather, there is one process of photosynthetic carbon metabolism that is the sum of the $C_2$ plus $C_3$ cycles. Less informative nomenclature based on names of investigators, such as Calvin/Benson or Hatch/Slack cycles, is slowly disappearing. Topics related to the $C_2$ cycle started at about the dates shown in Table 1, which also lists some events of my life. Metabolic pathways and enzymatic properties to support the $C_2$ cycle are in the references. Our recent report of an $O_2$ compensation point ($\Gamma$) during photosynthesis (10) has extended our viewpoint, but because it has not yet been extensively debated, those parts of this chapter addressing this are more speculative. A more extensive review of the $O_2$ $\Gamma$ may be found in a manuscript in preparation with Erwin Beck. For 50 years, investigators have studied glycolate and P-glycolate synthesis and its function, regulation, and inhibition. I am extending this with speculation on regulation of global $CO_2$ and $O_2$ concentrations. It will not be easy to overcome 150 years of dogma that photosynthesis is only $O_2$ evolution and $CO_2$ fixation (it is $O_2$ and $CO_2$ exchange), and the physiological impact that Rubisco is also an oxygenase. Most difficult of all is to understand that at the recent low atmospheric $CO_2$ and high $O_2$ about half of photosynthetic energy has been used by the $C_2$ cycle.

Photosynthesis has been divided into two parts: light reactions for ATP synthesis plus NADP reduction with $O_2$ evolution and photosynthetic carbon metabolism to use the ATP and NADPH. There are two parts to carbon metabolism as initiated by the dual activities of ribulose bisphosphate carboxylase/oxygenase (Rubisco). It catalyzes carboxylation of ribulose bisphosphate (RuBP) with $^{14}CO_2$ to form two molecules of carboxyl labeled 3-P-glycerate of the $C_3$ reductive photosynthetic carbon cycle. Rubisco is also an oxygenase that catalyzes oxidation of RuBP by addition (fixation) of an $^{18}O_2$ to RuBP to form one carboxyl [$^{18}O_2$]P-glycolate and one 3-P-glycerate of the $C_2$ oxidative photosynthetic carbon cycle. The $C_2$ and $C_3$ cycles coexist and together are photosynthetic carbon metabolism (Figure 1). The ratio of $CO_2$ and $O_2$ con-

centrations in the chloroplast determines the flow of carbon between the two cycles.

At low global $CO_2$ equilibrium level of <0.03% $CO_2$ and ~21% $O_2$, $CO_2$ fixation by the $C_3$ cycle in $C_3$ plants is about three times more than $CO_2$ production by the $C_2$ cycle. Thus we have net $CO_2$ fixation and $O_2$ evolution. However, the $C_2$ cycle uses about three times more energy per $CO_2$ turnover. Thus, in air with high light intensity nearly equal amounts of photosynthetic energy are used by the $C_3$ cycle for net photosynthesis as are consumed by the $C_2$ cycle (2, 9). A lower limit of $CO_2$ and an upper limit of $O_2$ are compensation points ($\Gamma$), beyond which net photosynthesis is zero and plants senesce from excess photooxidation of storage carbohydrates by oxygenase activity of Rubisco and the $C_2$ cycle. The $O_2$ $\Gamma$ with 220 ppm $CO_2$ at 20°C is about 23%

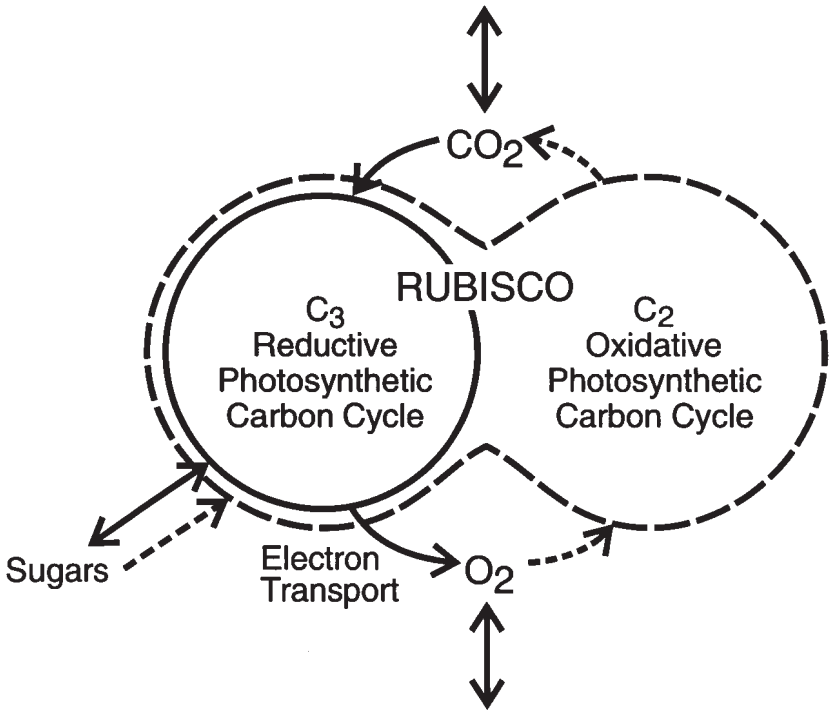


*Figure 1* The $C_2$ and $C_3$ cycles of photosynthetic carbon metabolism. This scheme (9, 10) emphasizes that the two cycles coexist, that both represent about equal parts of the process, that carboxylase and oxygenase activities of Rubisco initiate both, and that there is just one $CO_2$ and one $O_2$ pool. Although the $C_2$ cycle has been called photorespiration, it is not a separate process. Rather, the two cycles create a necessary balance during photosynthesis for net $CO_2$ and $O_2$ exchange.

**Table 1** Glycolate oxidase, glycolate synthesis, glycolate pathway, and other related events of my life

| | |
|---|---|
| 1920 | O Warburg published that $O_2$ inhibited photosynthesis. That year I was one year old in Twin Falls, Idaho |
| 1930–1950 | Photosynthetic investigators considered $O_2$ inhibition of electron transport |
| 1940–1941 | Ruben's search for the first product of $^{11}CO_2$ fixation. My BS, Department of Chemistry, University of California, Berkeley |
| 1941–1943 | University of California, Davis, Department of Viticulture and Enology, a diversion for me to use the carbon products of photosynthesis |
| 1943–1946 | World War II, Captain, US Air Force Intelligence |
| 1946–1949 | Glycolate oxidase, PhD thesis with RH Burris, University Wisconsin |
| 1950 | In Calvin's group with Benson and Bassham. $^{14}CO_2$ fixation by the $C_3$ cycle and [$^{14}$C] glycolate, glycine, and serine found to be major early products |
| 1950–1952 | US Atomic Energy Commission grants for plant science. Glycolic oxidase light activation at USDA, Beltsville |
| 1952–1958 | Glycolate metabolism and glycolate excretion by algae. Biology Division at Oak Ridge National Laboratory, Organizer of Gatlinburg Photosynthesis Conferences |
| 1958–1962 | Professor of Biochemistry Michigan State University. Glycolate pathway; P-glycolate phosphatase. Concept of photorespiration developed by many investigators |
| 1960–1968 | The $CO_2$ compensation point. $C_4$ cycle and $C_4$ plants. Construction of biochemistry building with RG Hansen and WA Wood |
| 1968–1975 | P-glycolate synthesis by oxygenase activity of Rubisco. Glycolate metabolism in peroxisomes from leaves or liver |
| 1975 | The $C_2$ oxidative photosynthetic carbon cycle. Algal $CO_2$ concentrating processes. Effect of increasing atmospheric $CO_2$ on plants |
| 1989 | Professor Emeritus—too soon |
| 1996 | The oxygen compensation point. Glycolate metabolism in chloroplasts. Regulation of the ratio of atmospheric $CO_2$ and $O_2$ by Rubisco |

$O_2$ for $C_3$ plants (10). The ratio of $CO_2$ and $O_2$ concentration in the chloroplast limits net photosynthesis, as well as sets the $CO_2$ and $O_2$ atmospheric ratio on a short-term basis. Long-term, i.e. centuries, geological global carbon and $O_2$ cycles provide sources of $CO_2$ and sinks for $O_2$, a process that slowly establishes atmospheric equilibrium levels. Besides the ratio of $CO_2$ and $O_2$ concentrations, many other plant growth factors also determine rate of net photosynthesis. These are not integrated into this summary, but among them are temperature, light intensity, water, nutrient, stomata openings, canopy coverage, processes for concentrating $CO_2$ and exclusion of $O_2$, membrane diffusion, and geological storage reservoirs of $CO_2$ and $O_2$. Changes in any of these alter

total photosynthesis, but the short-term $CO_2$ and $O_2$ equilibria are based on kinetic properties of the immense amount of Rubisco.

From the time of the discoveries of $O_2$ inhibition of $CO_2$ fixation, glycolate oxidase, photorespiration, oxygenase activity of Rubisco, and leaf peroxisomes (Table 1), a continuing question has been their purpose. These phenomena occur at 21% $O_2$ and increase at higher $O_2$ levels, as if they were the result of $O_2$ toxicity. Many of us sought ways to reduce this $O_2$ inhibition to increase crop yield. So far there has been no evidence that research targeted at modifying Rubisco has been agronomically successful. This may be because $O_2$ inhibition of $CO_2$ fixation is now an essential, environmental part of photosynthesis. In addition, incomplete data have limited our understanding, and even now we are just publishing about the $O_2$ $\Gamma$ (10) and a second glycolate oxidizing system in chloroplasts (1). Earlier, G Lorimer & J Andrews pointed out that oxygenase activity of Rubisco was probably unavoidable because there are no binding sites for $CO_2$ or $O_2$ substrates, and I am adding that this dual specificity is an essential part of photosynthetic carbon metabolism. The $O_2$ $\Gamma$ is a consequence of oxygenase activity of Rubisco and is an upper limit on the permissible atmospheric $O_2$ level for plant growth, just as the $CO_2$ $\Gamma$ limits lowering the atmospheric $CO_2$.

## *My Start in Low $CO_2$*

The story about $O_2$ regulation of photosynthesis began in 1920, a year after I was born, when Otto Warburg published that air (21% $O_2$) inhibited $CO_2$ fixation by algae. I grew up on a farm at Twin Falls, Idaho, where recreation was fishing and hunting in canyons along the Snake River. From then till well past my graduate school, photosynthesizers discussed how $O_2$ might oxidize chloroplast electron transport components. Rabinowitch wrote that if $O_2$ inhibition effected respiration, all their hypotheses were off. It did, because $O_2$ inhibition of $CO_2$ fixation is the $C_2$ cycle, which is glycolate synthesis and metabolism.

The small rural grade school that I attended, at first by a horse-drawn covered wagon as a school bus, taught only the three basics and baseball, but my mother supplemented the basics with nightly drills around kerosene lamps. I read Zane Grey, but I never learned to spell well or master languages, even later after suffering through Latin, French, and German. I must have been a disappointment to my mother, who had a Master's degree in English from the University of Chicago. I am not an extrovert, but I became a daydreamer from sitting all day on a farm cultivator. I do best when talking about science.

On the farm, my father trained me to do irrigation and stack hay. It was somehow assumed that we would go to college, and I, being the oldest, led all four of us to major in science. In the first two years at the University of Southern Idaho in Pocatello, I remember best Sunday hikes up the canyons into the mountains. After two years, my father suggested that if I returned home, he would buy a sheep ranch for us near Ketchum or Sun Valley. That temptation has often been remembered, but I went instead to the University of California, Berkeley, where I was a B+ student with that inferiority complex. I deviated once from chemistry, when I took an intensive course in biochemistry for premed majors and pulled my only C. Thus, I was challenged. At this time, the first radioactive, $^{11}CO_2$ photosynthetic experiments were done by Ruben (until he was killed by phosgene gas) while I was in a nearby lab. Melvin Calvin, another assistant professor in the Chemistry Department, replaced him. At graduation in 1941, I was told to find employment at Shell Oil Research down by the Bay, near Berkeley. Upon going there, the aromas from their labs and mud flats made me long for a sheep ranch in Idaho. Returning from that interview, I stopped at the student employment office on campus and learned that the Head of Viticulture and Enology at UC Davis was there looking for an organic chemist. Within half hour I had a job, conceptually nearer the farm, to analyze brandy for $150 a month. My first publications were in the *Journal of Food Research*, on pH and tannins. Maynard Amerine, Professor of Enology and American's foremost wine connoisseur, invited AD Webb and me to live with him in Davis while he taught us wine tasting. Until then I had been a teetotaler, but suddenly I was a wine and brandy expert. My parents may have been in shock, but they said nothing.

At that time (about 1940), the atmospheric $CO_2$ level was around 300 ppm. It had been first measured in 1890 at about 290 ppm and concern was expressed even then that it might be rising. In graduate school in the late 1940s we used 303 ppm as the $CO_2$ level. In 1957, when the $CO_2$ level was 315 ppm, NSF began funding measurements of the $CO_2$ concentrations atop Mauna Loa volcano in Hawaii. Atmospheric $CO_2$ is now about 380 ppm. I use $CO_2$ levels in Arctic ice cores to indicate an atmospheric $CO_2$ equilibrium for the past 165,000 years that oscillated around $235 \pm 45$ ppm with 21% $O_2$. This seems to have been an equilibrium whose highs of 280 ppm $CO_2$ corresponded with interglacial periods and whose lows around 190 ppm occurred during glacial periods. I hope this chapter will promote discussion about whether competition between atmospheric $CO_2$ and $O_2$ levels for Rubisco catalysts during photosynthesis limited $CO_2$ level on the downside and maintained global $O_2$ level at 21%. Now we are in the midst of a mammoth atmospheric experiment in which the $CO_2$ concentration has been raised about 50% to 380 ppm. At this

current $CO_2$ level, the permissible $O_2$ level from an equilibrium based on the specificity of Rubisco could rise over a long period of time to around 28% $O_2$ (10).

In the Chemistry Department at Berkeley my senior thesis for M Randall, coauthor of the textbook on physical chemistry, listed the literature citations in Beilstein and Chem Abstracts on light-activation of silver halides. In 1942, as my draft number rose toward the top, I applied to the Air Force for officer training as a photographic expert on the basis of that compilation. After boot camp in Florida and classes at Yale University, assignments were New England photo lab at Bradley Field, Connecticut; photo intelligence school at Orlando, Florida; briefing B-24 crews at Muroc Air Force Base in Southern California; air force intelligence in the Philippines (purple heart); and Japan. This captain had traveled a long way from that sheep ranch in Idaho. Following the war, I settled down as a graduate student in biochemistry at the University of Wisconsin in Madison with RH Burris as PhD advisor, mentor, and long-time friend. He started me on polyphenol oxidase, which seemed to be responsible for browning of potato tubers. $O_2$ and $CO_2$ gas exchange measurements were run with a Warburg apparatus with 18 manometers. Today, Warburg analyses have fallen into complete oblivion, because they are deemed too complicated. Instead, $O_2$ changes are measured one at a time in an $O_2$ electrode, and $CO_2$ concentrations are assayed by an IR analyzer. For uptake of $^{14}CO_2$ and metabolism of $^{14}C$-labeled substrates, Warburg flasks and Thunberg tubes have some distinct advantages. After I started graduate school, Burris, one of the authors of *Manometric Techniques*, invented the circular Warburg, and I was his first graduate student to use it. It was a real advancement to sit in one place while rotating and reading the rack of oscillating manometers.

I have been helped greatly by my brother, Bert, and sister, Marian. Bert and I shared the same room at home and at college and took the same courses. He became an expert on synthesis and use of $^{14}C$-labeled compounds in biological research. He made 1- and 2-[$^{14}C$]glycolates, which I used to determine the glycolate pathway. M Calvin felt that glycolate was labeled from $^{14}CO_2$ via the sugar phosphates of the $C_3$ cycle (he was right), and so glycolate was relegated to the discard heap. Thanks! This left me the other half of photosynthetic carbon, the $C_2$ cycle.

## *The $C_2$ Cycle*

In the 1950s, glycolate and photorespiration topics were not mainstream, and we had to struggle to be heard. At the Brookhaven Photosynthesis Symposium

of 1963, Bessel Kok put his arm around me after my talk outlining reactions of the glycolate pathway (4) and said, "Ed, why keep working on that glycolate? You know it is not an early product during photosynthesis. There are many other more important things to do." Fortunately, I did not follow his advice.

My traditional biochemical research approach has been first to find and characterize enzymes and second to place them in metabolic pathways by studies with isotopically labeled substrates. A third approach, starting in the 1960s, was to use mutants to confirm or predict biochemical pathways. $C_2$ cycle mutants in Arabidopsis were developed by CR Somerville. It was gratifying that reactions of the $C_2$ pathway, as determined earlier, were confirmed with several mutants. We can now speculate that failure to isolate a mutant of peroxisomal glycolate oxidase is consistent with alternate glycolate metabolism in the chloroplast (1). The techniques of molecular biology became available as we worked on the algal $CO_2$ pump. For a complete research program, all four approaches should be used.

In 1948, Carl Clagett finished his PhD thesis with RH Burris on glycolate oxidase, and I continued my PhD research on this new oxidase, function unknown. Glycolate ($CH_2OH$-COOH) is oxidized with $O_2$ uptake to glyoxylate (CHO-COOH) and $H_2O_2$. Glyoxylate was trapped as a hydrazone or oxidized to $CO_2$ and HCOOH depending on the amount of catalase remaining to remove $H_2O_2$. From the start we thought that a glycolate/glyoxylate system might be a terminal oxidase to waste energy, which was then a popular concept. For that, I Zelitch and S Ochoa in 1957 characterized a glyoxylate reductase, which turned out to be the same as a glycerate dehydrogenase isolated in 1956 by B Vennesland. From our studies in the 1960s and 1970s this enzyme became known as hydroxypyruvate reductase, because it had a low $K_m$ of 10 μM for hydroxypyruvate ($CH_2OH$-CO-COOH), but a high $K_m$ of about 500 μM for glyoxylate, which was rapidly transaminated to glycine instead. However, possible peroxidation of glyoxylate and hydroxypyruvate by $H_2O_2$ has been considered numerous times by others. Conceptually, the $C_2$ cycle is a highly regulated, complex, terminal oxidase system involving extensive metabolic reactions to use excess photosynthetic energy, but it is not two, unregulated, coupled reactions of an oxidase and a reductase. For 20 years these enzymes were considered to be soluble or cytosolic, and it was not until 1968 that we realized that their cellular location was in the fragile, single-membrane-bound peroxisomes (5, 6).

The glycolate pathway part of the $C_2$ cycle is channeled irreversibly toward glycine and serine formation (2–7, 11). Enzymatic reactions by Rubisco and P-glycolate phosphatase in the chloroplast, glycolate oxidase and glyoxylate:glutamate aminotransferase in peroxisomes and glycine decarboxylase in

mitochondria are physiologically irreversible, consecutive reactions that start carbon flow around the $C_2$ cycle. The glycolate pathway only occurs in light because ATP and NADPH are needed to regenerate RuBP for the $C_2$ and $C_3$ cycles. When continuing around the $C_2$ cycle, serine hydroxymethyltransferase in mitochondria converts two glycines to one serine, $NH_3$, NADH, and a $CO_2$. An aminotransferase in peroxisomes converts the serine into hydroxypyruvate, which is reduced to glycerate. I consider these reactions as a reversible, glycerate pathway that can occur in light or darkness. $^{14}C$ metabolic studies with WA Wood showed that 3-carbon serine or glycerate were condensed to a 6-carbon hexose and ended up in sucrose. Because glycerate kinase is in chloroplasts, we assumed that the 3-P-glycerate entered that pool shared with the $C_3$ cycle. If you think the $C_2$ cycle is complex, so does everyone else, and at about this point many elect to disregard further the complete $C_2$ cycle. To consider the whole cycle, one needs to add: (*a*) regeneration of RuBP from 3-P-glycerate or from refixing $CO_2$ or from storage carbohydrate via the pentose phosphate pathway (a part of the $C_3$ cycle); (*b*) refixation of $NH_3$ in the chloroplast; (*c*) an aspartate:malate shuttle for balancing reducing equivalents among cell compartments; and (*d*) a glycolate/$H^+$ and/or glycolate/glycerate exchange, the triose phosphate shuttle, and glutamate transport for movement across chloroplast membranes.

Step-wise details of the glycolate pathway part of the $C_2$ cycle were elaborated in the 1950s and 1960s with B Rabson, E Jimenez, P Kearney, F Snyder, K Richardson, J Hess, G Orth, W Bruin, WT Chang, and A Baker. Reactions in the leaf peroxisomes and other organelles were first investigated in the 1970s with A Oeser, C Scharrenberger, T Kisaki, W Chang, D Rehfeld, R Yamasaki, R Gee, P Gruber, S Federick, Y Nakamura, J Hanks, and B Ho. Generally, each person concentrated on one phenomenon or step of the $C_2$ cycle by isolating and characterizing enzymes involved. For example, the properties and physiological role of P-glycolate phosphatase and P-glycerate phosphatase were investigated in the 1960s and 1970s with P Kearney, K Richardson, Y Yu, D Anderson, D Randall, J Christeller, and D Husic.

## *Rubisco: Phosphoglycolate and Glycolate Biosynthesis*

Regulation of one enzyme for one reaction can be complicated, but regulation at a metabolic branch point by an enzyme with dual activities, as Rubisco, is indeed complex. Rubisco has a binding site for RuBP and for an activating $CO_2$ but has no known binding site for its other substrates, $CO_2$ and $O_2$. Thus catalysis is very slow and dependent on $CO_2$ and $O_2$ concentrations in the chloroplast. Our emphasis has been on understanding glycolate biosynthesis

and metabolism, which developed into the $C_2$ cycle and then later into regulation of photosynthetic carbon metabolism relative to the $CO_2$ to $O_2$ ratio. More recent topics have dealt with increasing the $CO_2$ to $O_2$ ratio from the atmosphere by $C_4$ plants and the algal $CO_2$ and $HCO_3^-$ pumps.

In my lab, the 1970s was a decade of productive research on Rubisco and on peroxisomes with funds from NSF and NIH. Properties and regulation of Rubisco were studied with G Lorimer, J Andrews, J Pierce, C Peach, F Ryan, S McCurry, N Hall, R Gee, R Houtz, RM Mulligan, K Cook, and others (2, 3, 5–7, 11). The oxygenase activity of Rubisco in vivo has been underestimated by [$^{14}$C] glycolate formation during $^{14}CO_2$ fixation or by $O_2$ uptake. Because of $O_2$ competition, the carboxylase $K_m(CO_2)$ increases from ~12 μM $CO_2$ in nitrogen to 26–42 μM $CO_2$ in air (21% $O_2$). $V_{max}$ with high $CO_2$ and air does not change, but high enough $CO_2$ concentrations (perhaps 1000–1500 ppm) for $V_{max}$ are not available in vivo except in $C_4$ plants or algae with activated DIC pumps. Thus, in vivo activity of the carboxylase in a $C_3$ plant is decreased by the $O_2$ level in air. Likewise, $CO_2$ inhibits the oxygenase activity of activated Rubisco, but exclusion of $CO_2$ from oxygenase assays is hard or impossible to do. Activation of both activities of Rubisco requires a high activating $CO_2$ concentration of 27 μM $CO_2$, yet this much $CO_2$ inhibits the oxygenase. If $CO_2$ is removed to measure the oxygenase, the enzyme inactivates. DB Jordan & WL Ogren devised an isotopic ($^{14}$C and $^{32}$P) procedure for simultaneous chromatographic measurement of 3-PGA and P-glycolate, and from them calculated the $K_m$ and $V_{max}$ and a substrate specificity factor to compare Rubisco from various sources. The factor is approximately 80 for Rubisco from $C_3$ and $C_4$ plants, 60 for unicellular green algae, and from ~48 to 9 for photosynthetic bacteria. $CO_2$ activation of Rubisco is not competitive with $O_2$, because the activating $CO_2$ forms a $HCO_3^-$ lysine complex to structure the active site. There is little conjecture on Rubisco inactivation in vivo at low $CO_2$. With increasing $CO_2$ concentration, the oxygenase activity of Rubisco will be more fully activated, and the storage carbohydrates can be photo-oxidized by the $C_2$ cycle at high $O_2$ levels. On the other hand, at low $CO_2$ concentration of the $CO_2$ Γ of a $C_3$ plant, both activities of Rubisco may be low, because the enzyme may not be fully $CO_2$ activated. Fixation of $HCO_3^-$ by phosphoenolpyruvate carboxylase (PEPC) is not $O_2$ sensitive and seems to account for much of the dissolved inorganic carbon (DIC) fixation even by $C_3$ plants, because the $CO_2$ to $O_2$ ratio decreases at either the $CO_2$ or $O_2$ Γs.

Voluminous literature over the past 50 years on photorespiration and photosynthetic carbon metabolism contains proposed systems for glycolate synthesis and metabolism and energy balances. Only the oxygenase of Rubisco accounts for all known facts about glycolate synthesis and photorespiration.

An early concern was rapid formation of uniformly [$^{14}$C] glycolate during $^{14}CO_2$ fixation in light. This led to proposals for an alternate $CO_2$ fixation pathway involving condensation of two $CO_2$. Because amounts of glycolate were not generally measured, it was not emphasized that S.A. of glycolate was much less (<25%) than S.A. of $^{14}CO_2$. In addition, [$^{14}$C] glycolate was labeled with $^{14}CO_2$ slower than intermediates of the $C_3$ cycle. Later we showed that glycolate is formed photosynthetically in light, even in absence of $^{14}CO_2$, and of course, the S.A. had dropped to zero. Thus, glycolate synthesis is a photosynthetic, light-dependent photo-oxidation of carbohydrates, whether RuBP comes from newly fixed $^{14}CO_2$ in the $C_3$ cycle or from storage reserves. Carbons 1 and 2 of RuBP are uniformly labeled during $^{14}CO_2$ fixation and form uniformly labeled [$^{14}$C]P-glycolate when RuBP is oxidized by the oxygenase. Calvin's group labeled the first major $^{14}$C product on their paper chromatograms as PGA, which can stand for and contained 3-P-glyceric acid and 2-P-glycolic acid. My contribution to that era in Calvin's lab was made the first week I got there in 1950. These chemists were trying to acid hydrolyze $^{14}$C-labeled phosphate esters from their chromatograms, but this produced too much salt for rechromatography. As a biochemist, I suggested hydrolysis by a pinch of phosphatase, Polidase S.

A second major requirement for glycolate synthesis during photosynthesis is that one $O_2$ is incorporated into its carboxyl group, which, in turn, leads to carboxyl $^{18}$O-labeled glycine and serine. P-glycolate synthesis by Rubisco is enhanced with higher oxygen or with decreasing $CO_2$ concentrations. $K_m(O_2)$ for the oxygenase activity of Rubisco has been reported between 260 and 400 μM, or about the $O_2$ level in aerated water. $CO_2$ to $O_2$ ratio becomes the governing factor for the amount of glycolate synthesis relative to net $CO_2$ fixation.

Papers in the 1960s also proposed glycolate synthesis by peroxidation of carbohydrates formed during photosynthesis. Specifically, transketolase in the $C_3$ cycle transfers the top two carbon atoms of sedoheptulose-7-P or xylulose-5-P to its cofactor, thiamine pyrophosphate, to form a $C_2$ complex, which can be nonenzymatically oxidized by $H_2O_2$ to form glycolate. This reaction, however, would not incorporate $^{18}O_2$ into the carboxy group nor form P-glycolate. In 1965, an NIH sabbatical fellowship took me to Freiburg, Germany, with H Holzer, an expert on transketolase in yeast. Angelica Oser assisted me in countless Warburg measurements of $O_2$ uptake by isolated spinach chloroplasts. We found much $O_2$ uptake without a time lag, upon adding P-glycolate or glycolate. She continued on this puzzle at Michigan until we heard C de Duve and H Beever lecture on peroxisomes and realized that our chloroplast preparations were contaminated with peroxisomes. The transketolase hypothe-

sis was dropped, and we became experts on leaf peroxisomes. My associates for 30 years afterward know that I never ceased to wonder how chloroplasts could oxidize P-glycolate as fast as glycolate without a time lag to hydrolyze it to glycolate. Never ignore data. The explanation may be in our recent paper (1) on an alternate system in chloroplasts, which oxidizes either P-glycolate or glycolate.

## *$O_2$ and $CO_2$ Compensation Points*

By decreasing the $CO_2$ concentration while the $O_2$ level remained constant at 21%, J Decker in the 1950s found that at about 50 ppm $CO_2$, net $CO_2$ uptake by $C_3$ plants had declined to zero. This has been called the $CO_2$ $\Gamma$ in 21% $O_2$. At lower $CO_2$ levels in light there was $CO_2$ evolution, which became greater than dark respiration, and the $C_3$ plants senesced. The $CO_2$ $\Gamma$ increases with increasing $O_2$ concentrations (10). With a given level of $O_2$, there is a lowest concentration of $CO_2$ at which net photosynthetic $CO_2$ exchange is zero, and with even lower $CO_2$ or higher $O_2$, net $CO_2$ evolution occurs. In a simplification, the $CO_2$ $\Gamma$ has been considered as the $CO_2$ concentration with a given level of $O_2$, when $CO_2$ evolution from the $C_2$ cycle equals the rate of $CO_2$ fixation by the $C_3$ cycle. Historically, the $CO_2$ $\Gamma$ became well known in the 1960s from an unsuccessful search for $C_4$-like varieties of $C_3$ wheat and soybean plants in closed, survival chambers with $C_4$ corn plants. With funds from Union Carbide (D Manning), W Smith and HS Ku with us likewise surveyed unsuccessfully hundreds of compounds with $C_3$ plants for altering the $CO_2$ $\Gamma$ to see if they differentially effected the dual activities of Rubisco (notebooks of unpublished data). After that failure we became involved with processes to alter the $CO_2$ to $O_2$ ratio and thus the $C_2$ and $C_3$ cycles.

The immense $O_2$ level in air does not significantly vary, but small changes in the very low $CO_2$ level would alter the $CO_2$ to $O_2$ ratio and should change Rubisco reactions. Since $O_2$ diffuses extremely rapidly out of the leaf, $O_2$ exclusion does not appear to exist except for the thick-walled bundle sheath cells of $C_4$ plants that separate Rubisco inside from $O_2$ generated in the chloroplasts of the mesophyll cells. Four processes are known to change the $CO_2$ concentration around Rubisco. These are the $C_4$ cycle in $C_4$ and CAM plants, algal DIC concentrating processes, altering atmospheric $CO_2$ concentrations, and carbonic anhydrase in the chloroplast to accelerate $HCO_3^-$ conversion to $CO_2$. The algal $CO_2$ or $HCO_3^-$ pumps appear to directly import DIC, and although less is known about them, they should be simpler than the $C_4$ cycle. The algal DIC pumps use photosynthetic energy and are suppressed by high $CO_2$. Our group in this area has included J Moroney, D Husic, DW

Husic, A Goyal, Y Shiraiwa, J Thielman, S Dietrick, R Togasaki, and B Wilson.

The $CO_2$ Γ of $C_4$ plants is ~2 ppm $CO_2$. By concentrating $CO_2$ and partially excluding $O_2$ from bundle sheath cells of $C_4$ plants, the oxygenase activity of Rubisco is suppressed. Furthermore, $C_4$ plants initially fix $HCO_3^-$ by PEPC, which has a much lower $K_m(HCO_3^-)$ than the $K_m(CO_2)$ for Rubisco. In addition, at pH > 8, there is at least a 100-fold higher concentration of $HCO_3^-$ than $CO_2$. Important, but unrecognized earlier, is that $HCO_3^-$ fixation by PEPC is not inhibited by $O_2$! No wonder $C_4$ plants are more $CO_2$ efficient at low $CO_2$ or high $O_2$. Part of photosynthetic energy used by the $C_2$ cycle in $C_3$ plants is used for the $C_4$ cycle. An ecologically exciting part of this $C_4$ story (9) is that as long as atmospheric $CO_2$ level remains less than ~380 ppm $CO_2$, the $C_4$ plant has been more $CO_2$ efficient than $C_3$ plants. However, above ~380 ppm $CO_2$, the $C_4$ plant is nearly light saturated and does not further increase its rate of $CO_2$ fixation with more $CO_2$, because it must use energy for its $CO_2$ pump to get $CO_2$ to Rubisco in the bundle sheath cells. At ~380 ppm $CO_2$, $C_3$ and $C_4$ plants are about equal in photosynthetic efficiency for $CO_2$ fixation. As $CO_2$ continues to rise, $C_3$ plants will increase their $CO_2$ fixation rate with the excess energy that they no longer use on the $C_2$ cycle, and become more efficient than $C_4$ plants. This change has occurred in a geological moment of one century, as $CO_2$ level has increased from 280 to 380 ppm. From now on with further increases in $CO_2$, the $C_3$ plant should be photosynthetically superior to a $C_4$ plant.

With all the work over 35 years on $C_4$ plants and $CO_2$ Γ and realization that Rubisco and the $C_2$ cycle involved $O_2$ uptake, you can ask what the reasons were that no one found an $O_2$ Γ to match a $CO_2$ Γ. After all, Rubisco is both a carboxylase and an oxygenase. First, we have all considered photosynthesis as only $CO_2$ fixation. To do an experiment to measure the $O_2$ Γ, the $CO_2$ concentration must be held constant, and small changes in the high $O_2$ concentration must be measured over long periods. This is technically difficult and requires an $O_2$ leak-proof chamber. Second, when the $CO_2$ level is held constant at 220 or 350 ppm, rates of $CO_2$ fixation are reduced between 5% $O_2$ up to 100% $O_2$, but are never completely inhibited. To repeat: because of $O_2$ inhibition, rate of net $CO_2$ fixation and $O_2$ evolution decreases with increasing $O_2$, but not to a zero rate of $CO_2$ fixation. Investigators of $^{18}O_2/^{16}O_2$ exchange likewise either used low $O_2$ or found increasing $O_2$ exchange with increasing $O_2$ or decreasing $CO_2$, but never zero $CO_2$ fixation. Thus, it was implied that there was no photosynthetic compensation point at high $O_2$. Wrong! Although there is no $CO_2$ Γ with higher $O_2$, there is an $O_2$ Γ based on zero net $O_2$ change (10), which also limits net photosynthesis and plant growth just as much as the $CO_2$

Γ at low $CO_2$. The $O_2$ Γ may be thought of as that $O_2$ concentration when the rate of $O_2$ evolution has decreased to the rate of $O_2$ uptake in a given $CO_2$ concentration.

I was honored by a Senior Scientist award from the German Humbolt Foundation. First, I spent six months with H Senger at the University of Marburg examining with J Thielman the DIC pump in *Scenedesmus.* Then at the University of Bayreuth, with Erwin Beck, we pondered why there was no $O_2$ Γ for plants. A photosynthetic chamber was built with controls for light, temperature, and $CO_2$ and $O_2$ concentrations. While holding $CO_2$ constant, the rate of $O_2$ change was measured at different $O_2$ concentrations from 2 to 90% with young tobacco or spinach plants. With increasing $O_2$, net rate of $CO_2$ fixation and of $O_2$ evolution decreased as expected from $O_2$ inhibition. With 220 ppm $CO_2$, the $O_2$ evolution had decreased to zero at 23% $O_2$ or with 350 ppm $CO_2$ at 27% $O_2$ (10). These are the $O_2$ Γs, when net rate of $O_2$ exchange at a fixed $CO_2$ level and temperature is zero. Nevertheless, at the $O_2$ Γ, the rate of $CO_2$ fixation had only fallen ~60% because of $O_2$ inhibition, although $O_2$ evolution had fallen 100%. With $O_2$ levels over the $O_2$ Γ, net $O_2$ uptake occurred (no $O_2$ evolution), and at 30 to 40%, $O_2$ rates of $O_2$ uptake increased to rates nearly equal to $CO_2$ uptake at low $O_2$. At first, one might say "impossible if photosynthesis is occurring." This were as if at low $O_2$ and/or sufficient $CO_2$, $CO_2$ fixation was the acceptor of the photosynthetic energy, but at high $O_2$ over the $O_2$ Γ, $O_2$ was the sole acceptor of photosynthetic energy. Above the $O_2$ Γ, plants slowly senesced. Our first conclusion is that an $O_2$ Γ exists and limits plant growth not far above present 21% $O_2$ with past low levels of $CO_2$. At a low $CO_2$ to high $O_2$ ratio, $O_2$ fixation by Rubisco functions as the alternate electron acceptor during photosynthesis rather than $CO_2$. GC-MS analysis of the products in tobacco leaves after several hours in high $O_2$ over the $O_2$ Γ found a big increase in malate. So E Beck and I proposed that increasing $O_2$ inhibition of the carboxylase of Rubisco reached a point where net photosynthesis, when measured as $O_2$ evolution, reached zero. Continued fixation of $CO_2$ could be due to $O_2$-insensitive, $HCO_3^-$ fixation by the abundant PEPC in $C_3$ plants. The products, oxaloacetate and malate, are not reduced by the $C_3$ cycle to carbohydrate with accompanying $O_2$ evolution. For $O_2$ evolution by photosynthetic electron transport, NADP reduction must occur and be used for $CO_2$ reduction by the $C_3$ cycle. In the absence of enough $CO_2$ in air or the presence of too much $O_2$, net $O_2$ exchange in the light became $O_2$ uptake, and the plants senesced. The concept of an $O_2$ Γ opens up a new field dealing with photosynthetic limitation of the atmospheric $O_2$ levels, just as the $CO_2$ Γ limits $CO_2$ removal. Our research on this is being done after my retirement and without a grant. Unhappiness is at the lack of under-

standing, earlier, of all the physiological aspects of Rubisco and, now, at the lack of financial support for plant physiology research.

## *Photosynthetic Quotient and Quantum Efficiency*

Earlier studies on photosynthesis had decreed a photosynthetic quotient of one $CO_2$ fixed and one $O_2$ evolved from photolysis of water. As long as the $CO_2$ level was high, over ~1500 ppm, and/or the $O_2$ level low, the oxygenase activity of Rubisco would be suppressed. As the $O_2$ level increases toward 21% with low $CO_2$ or surpasses the $O_2$ Γ, a higher percentage of DIC fixation is for $HCO_3^-$ by $O_2$-insensitive PEPC. Because the complete $C_2$ cycle results in no net $CO_2$ or $O_2$ exchange as long as the $O_2$ level is below the $O_2$ Γ (7, 9), the $C_2$ cycle is normally a Hill process and only reduces the amount, but it does not change the ratio of one $CO_2$ uptake to one $O_2$ evolved. However, the quantum efficiency decreases from $O_2$ inhibition because of consumption of energy by the $C_2$ cycle. At $O_2$ levels above the $O_2$ Γ, which blocks the $C_3$ cycle, there is continued $CO_2$ fixation into malate and net $O_2$ uptake, so the ratio of $CO_2$ evolution to $O_2$ uptake becomes meaningless. Past avoidance of these problems is illustrated in a quote by my colleague, N Good, in his prefatory chapter in the *Annual Review of Plant Physiology* (1986, pp. 5–6):

> We succeeded by using isotopically labeled oxygen as the substrate for respiration, since the oxygen produced from water by photosynthesis is, of course, unlabeled. The problem was of considerable importance in those days because the great Otto Warburg had decreed that photosynthesis requires an immense uptake of oxygen, reconsuming three quarters of the photosynthetically produced oxygen. We succeeded in showing that Warburg was wrong....I have sometimes been asked how we came to miss the phenomenon of photorespiration since our instrument was ideally suited for detecting it. The simple answer is that we did not miss photorespiration at all. We observed it and adjusted conditions to eliminate it, using low concentrations of oxygen and high concentrations of carbon dioxide. We chose these conditions specifically to avoid a phenomenon that was already well known but irrelevant to our concerns. Photorespiration has been described many years before by Warburg, who called it "photocombustion."

Warburg was right for air with 21% $O_2$ and low $CO_2$. He worked with algae in small closed Warburg flasks in light, which led to rapid accumulation of high $O_2$, high pH, a low $CO_2/O_2$ ratio, and domination by the oxygenase activity of Rubisco for P-glycolate synthesis. I prize a letter in 1958 from Warburg congratulating us on discovering glycolate excretion by algae, because "its less reduced state would explain low quantum efficiency of 4." He then published that up to 80% of $CO_2$ fixed by *Chlorella* during photosynthe-

sis was glycolate. At the time, I had no explanation for this, but I now can speculate that the glycolate would have come from oxidation of storage carbohydrate by Rubisco and did not represent newly fixed and reduced $CO_2$. Thus, Warburg calculated a high quantum efficiency on much glycolate formation during low $CO_2$ fixation or $O_2$ evolution. These two examples are from past dogma by top scientists in photosynthesis. They did not know about the oxygenase activity of Rubsico nor consider photosynthesis to be both "$CO_2$ fixation and evolution" as well as "$O_2$ evolution and $O_2$ fixation."

## *Booby Traps with Algae*

Much research in photosynthesis has been conveniently done with algae. In order to use small amounts of $^{14}CO_2$ because of expense and radioactivity, cultures were used in small closed flasks. Rapid growth on 2 to 5% $CO_2$ suppressed the algal $CO_2$ pump so they were like a $C_3$ plant. When first put on low $CO_2$, $O_2$ accumulated as the $CO_2$ level dropped, and the $CO_2$ to $O_2$ ratio favored the $C_2$ cycle with glycolate and malate formation. This trapped Warburg and every one else. After I and P Zill published that algae specifically excrete glycolate, T Fogg, other marine biologists, and we visualized a photosynthetic glycolate symbiosis with flora in the ocean. However, in lakes mere traces of glycolate were much lower than expected, as the algae are adapted to low, free $CO_2$ and do not excrete much glycolate.

Unicellular green algae do not have peroxisomes or glycolate oxidase but rather appear to metabolize glycolate at the slow rate of ~1 μmole • mg $Chl^{-1}$ • $hr^{-1}$ by a mitochondrial glycolate dehydrogenase (work done with E Nelson, S Frederick, DW Husic, A Goyal, P Kehlenbeck, N Selph). However, when glycolate metabolism is blocked by an aminotransferase inhibitor (aminooxyacetate) for glyoxylate conversion to glycine, algae excrete at a rate of up to 15 μmole glycolate and some glyoxylate. This high excretion rate, which varies with the $CO_2$ to $O_2$ ratio, has been used to indicate the rate of glycolate production. For 20 years we have procrastinated about these data. Either we could not assay the mitochondrial glycolate dehydrogenase or there was another enzyme for oxidizing glycolate. Now we think the latter may be the case (1).

Leaf type peroxisomes are present in the multicellular charophyceae line of algae, which is the only line of algae that evolved into higher plants. In many other unicellular algae, these peroxisomal enzymes appear to be in their mitochondria. The small multicellular *Mougoetia* in the Charophyceae, as well as *Chara* and *Nitella*, have peroxisomes with enzymes for the $C_2$ cycle (8). Is there some special reason why leaf-type peroxisomes had to be present before higher plants could evolve? Rubisco is in all algae along with much P-glyco-

late phosphatase, as studied with D Husic and D Randall. The $K_m(O_2)$ for Rubisco oxygenase and for peroxisomal glycolate oxidase is about 260 μM, which is obtained by bubbling water with air, and both activities increase with higher $O_2$ (8). Thus, leaf type peroxisomes in green algae should not have functioned before the atmospheric $O_2$ increased (nor plants evolve?).

Before the above was known about algae, I proposed in 1970 a comparison of photorespiration in plants with that in marine algae. Up to that time, most work on glycolate had been done with plants or freshwater algae. NSF funded a three-month study of photorespiration by 20 plant physiologists and algologists aboard the *Alpha Helix* research vessel at Lizard Island on the Great Barrier Reef, before there were many tourists. This was a scientific trip that proved I had been right in not returning to the farm in Idaho. Our papers occupied an issue of *Australian Journal of Plant Physiology*, but we did not find much evidence of photorespiration or glycolate excretion. This resulted in turning my attention to $CO_2$-concentrating processes in algae. I need to emphasize how much such field research taught us bench scientists, and how it rewarded us for a lifetime of hard research.

Other topics that were discussed on the Barrier Reef were coral deposits and symbiosis between algae and the polyps for fixed nitrogen, fixed $CO_2$ (glycerol), and phosphate. $CaCO_3$ deposits represent immense reservoirs of $CO_2$ that have formed during algal photosynthesis, just as fossil fuels are reduced carbon deposits. Many algae produce an external carbonic anhydrase to accelerate the conversion of $HCO_3^-$ to $CO_2$ plus $OH^-$. As the $CO_2$ is fixed, the $OH^-$ titrates a second $HCO_3^-$ to $CO_3^=$, which forms insoluble salts (8). In an unbuffered culture of green algae in the light with $NaHCO_3$, the pH quickly rises to 8.5 to 9, and $CO_2$ fixation stops. When the pH is high, the $CO_2$ concentration is low and the algae turn to $O_2$ fixation with the excretion of glycolate, which acid ($Pk_a$ of 3.8) might neutralize some of the $HCO_3^-$ and $CO_3^=$. Carbonate deposits during photosynthesis are a sink for atmospheric $CO_2$, but these topics have not been pursued vigorously, and funds for work like our Great Barrier Reef trip are sadly not now in vogue.

## *What If? Is Photorespiration the Best Term?*

I tell this story to emphasize that Rubisco is both an oxygenase and a carboxylase and that $O_2$ and $CO_2$ fixation coexist. Remember that compounds of the $C_2$ cycle are not labeled by $^{14}CO_2$ as fast as in the $C_3$ cycle, and that $^{18}O_2$ labels the $C_2$ cycle but not the $C_3$ cycle. In 1940, Ruben used radioactive $^{11}CO_2$ and Calvin and colleagues from 1946 on used $^{14}CO_2$ to find the first products of photosynthetic $CO_2$ fixation. About 20 years later, G Lorimer, J Andrews, and I, using $^{18}O_2$ with GC-MS analyses, examined the first products

of $^{18}O_2$ fixation (P-glycolate, glycolate, glycine, and serine) as expected from the $C_2$ cycle. Now just suppose that Calvin's group could have used a radioactive isotope of oxygen or that my major professor, RH Burris, who had a home-made mass spectrometer at that time for $^{15}N$ analyses, had used $^{18}O_2$. The $C_2$ cycle might have been discovered first and be the photosynthetic carbon cycle. This story is not a criticism, Dr. Burris, just a lament. One reason the reverse did not happen was that during the preceding 150 years, photosynthesis was conceptually $CO_2$ fixation, and no one conceived of $O_2$ fixation. After all, photosynthesis evolves $O_2$, and measurements of net $O_2$ uptake in the light never occurred until now (10). Terms such as photodecomposition by O Warburg and photorespiration by J Decker, G Krotkov, W Jackson and R Volk, M Gibbs, I Zelitch, W Ogren, B Osmond, myself, and others were not considered as photosynthesis. $^{18}O_2/^{16}O_2$ exchange and $O_2$ as an energy acceptor was considered photorespiration and as a wasteful, protective mechanism when $CO_2$ was low. Photosynthesis was by dogma only $O_2$ evolution but not $O_2$ fixation. We must recognize that during photosynthesis, $CO_2$ and $O_2$ are both fixed and evolved (Figure 1) to arrive at net gas exchanges. Old dogma obscured $CO_2$ and $O_2$ exchange by the dual reactions of Rubisco. $^{14}C$-labeling and analyses of the components of the $C_2$ cycle greatly underestimated their amount (especially from unlabeled storage carbohydrate). The term photorespiration implies distinct processes for $O_2$ uptake and $CO_2$ evolution that are masked by refixation of $CO_2$ and $O_2$ evolution. Calculations that were made of the energy consumed by photorespiration were based on the glycolate pathway, but not on the complete $C_2$ cycle. Total energy consumed should have included RuBP oxidation by the glycolate pathway followed by $CO_2$ refixation and resynthesis of RuBP. Both $C_2$ and $C_3$ cycles require regeneration of RuBP by reactions of the $C_3$ cycle for continued cycling (Figure 1). The energy used for resynthesis of RuBP is a major cost of either cycle. In a complete turn of the $C_2$ cycle, there is as much $O_2$ uptake as $O_2$ evolution and as much $CO_2$ evolution as $CO_2$ refixation. This is the case normally when there is sufficient $CO_2$ for net $CO_2$ fixation and $O_2$ evolution. In a closed box when the $CO_2$ concentration is less than the $CO_2$ $\Gamma$, there is net $CO_2$ evolution, or when $O_2$ is higher than the $O_2$ $\Gamma$, there is net $O_2$ uptake in the light from oxidation of the carbohydrate reserve. Then the $C_3$ cycle is blocked, and $O_2$ fixation is an alternate energy consumer to $CO_2$ assimilation.

If the term photorespiration has been confusing to others, it has also misled investigators. I have used photorespiration as a crutch to refer to several related processes (1, 5, 8, 9, 11). It has been used for glycolate biosynthesis, glycolate pathway, peroxisomal respiration, $C_2$ oxidative photosynthetic carbon cycle, $O_2$ inhibition of photosynthesis, $^{18}O_2/^{16}O_2$ exchange, and the dark $CO_2$ burst.

The two processes of photosynthesis and photorespiration have been considered so different that we used two abbreviations for them, PS and PR, and two pools of $CO_2$ and two of $O_2$ that change in opposite manner. These facts miss the purpose and function of the $C_2$ cycle and the reason Rubisco is an oxygenase. Let us develop the realization that the oxygenase activity of Rubisco and the subsequent $C_2$ cycle are essential and equal parts of photosynthetic carbon metabolism as the $CO_2$ decreases or the $O_2$ increases. The combination of the $C_2$ and $C_3$ cycles provides a check and balance on net photosynthesis and atmospheric concentrations of $CO_2$ and $O_2$. Photosynthetic carbon metabolism has its own Hill process or alternate electron acceptor in $O_2$ fixation. Photosynthesis is self-limiting by balancing the atmospheric $CO_2$ to $O_2$ ratio within limits set by the specificity of Rubisco. At lower $CO_2$ or higher $O_2$ than the Γs, plants cannot grow. We all should be proud to be a part of the plant science community as these concepts evolve. This group includes plant physiologists and photosynthesizers who have investigated $CO_2$ fixation and the $C_3$ cycle, and $O_2$ fixation and the $C_2$ cycle. Although photosynthetic carbon metabolism, as a section or symposium topic, has disappeared from national biochemistry meetings, it flourishes at plant physiology meetings, International Photosynthetic Congresses, the Gordon Conferences, and other special symposia.

The question I am raising is whether the term "photorespiration" is appropriate to represent the oxygenase activity of Rubisco, because the oxygenase is as much a part of photosynthesis as is the carboxylase activity. It would be more informative to use "$O_2$ exchange or $CO_2$ exchange" during photosynthesis. In this chapter, I have also used the term "$O_2$ fixation" to parallel "$CO_2$ fixation." This could be confusing, because normally there is no measurable net $O_2$ fixation even though $O_2$ exchange as well as $CO_2$ exchange is occurring. Emphasis is needed in photosynthetic carbon metabolism that Rubisco is both a carboxylase for $CO_2$ fixation with $O_2$ evolution, as well as an oxygenase for $O_2$ fixation with $CO_2$ evolution. Both cycles consume photosynthetic energy. We need to comprehend that the two Rubisco reactions together establish a ratio of $CO_2$ to $O_2$ in the atmosphere that is based upon its specificity. The $CO_2$ to $O_2$ ratio only recently reached an atmospheric equilibrium, with the geological pools. Maintenance of this ratio is a primary result of photosynthetic $CO_2$ and $O_2$ exchange. We should not imply that the oxygenase activity of Rubisco is wasteful, unavoidable, undesirable, or a separate process. Instead, for the global $CO_2$ and $O_2$ balance, both the $C_2$ and $C_3$ cycles coexist and are essential. Understanding this will be difficult for every one—ecologists, geologists, and legislators. However, first scientists like us must agree on these interpretations.

## *Regulation of Atmospheric $CO_2$ and $O_2$ Levels*

Plant scientists are not geologists, yet our research is important to environmental regulation. Recognizing this, I asked JCG Walker, a leading geologist, to speak at my retirement symposium on the role of photosynthesis in regulating atmospheric $CO_2$ and $O_2$ levels. His paper (in Reference 12) is a superb summary of the global carbon and oxygen cycles. He used photosynthesis to supply the atmosphere with $O_2$ and to remove $CO_2$, but he concluded that otherwise photosynthesis has absolutely nothing to do with regulating the $CO_2$ and $O_2$ concentration in air. This conclusion indicates a need to understand one another's data. We need to consider the great mass of plants and algae and great differences in the kinetics of enzymatic reactions (seconds), such as Rubisco, versus geological times for nonphotosynthetic changes in the storage pools. Do rapid photosynthetic changes in low $CO_2$ concentrations alter the atmosphere $CO_2$ and $O_2$ ratio on a short time basis, while the nonbiological changes may take centuries? The Mauna Loa data on $CO_2$ show a short-term decrease in summer of about 15 ppm in atmospheric $CO_2$, even in the presence of $CO_2$ replacement from $CO_2$ pools in the global carbon cycle. This rapid photosynthetic $CO_2$ depletion must be replaced, otherwise the $CO_2$ level would quickly decrease to where plants would have little net photosynthesis in 21% $O_2$. Lowering of $CO_2$ much below past equilibria levels of $235 \pm 45$ ppm may not be possible for $C_3$ plants, because oxygenase of Rubisco would dominate in 21% $O_2$. The $O_2$ $\Gamma$ with lower $CO_2$ levels, rather than the extremely low $CO_2$ $\Gamma$ from PEPC, may be the short-term limiting factor in 21% $O_2$ for plant growth. Fortunately, now that $CO_2$ is rising, the oxygenase activity is decreasing relative to the carboxylase. I propose that the short-term $CO_2$ to $O_2$ ratio in the atmosphere over decades is set by the $O_2$ and $CO_2$ specificity of Rubisco, whereas the large storage reserves of carbon and oxygen more slowly alter these gases over many centuries. In the distant past, as long as there were low $O_2$ and/or high atmospheric $CO_2$ to lower the oxygenase of Rubisco, the $CO_2$ to $O_2$ equilibrium ratio from its specificity was not reached. Now our grand environmental experiment with increasing $CO_2$ may have started from a low $CO_2$ to $O_2$ equilibrium and is being conducted in the presence of high $O_2$ and rising temperatures. Considerable thought will be needed to evaluate whether or how fast an increase might occur in the $O_2$ level toward a higher $O_2$ $\Gamma$.

Photosynthetic investigators have justified their research as the basic process for plant growth for food, fuel, and fiber and $O_2$ for life. We have also expressed concern about increasing atmospheric $CO_2$ with an increase in the greenhouse effect. Another dimension with which we have not dealt is regula-

tion of atmospheric $O_2$ concentration or ratio of $CO_2$ and $O_2$. The $CO_2$ level of the past 165,000 years, as found in the ice cores, has shifted between 190 ppm to 280 ppm. The activity of Rubisco and distribution of $C_3$ and $C_4$ plants should have varied within this $CO_2$ to $O_2$ range because of changes only in the $CO_2$ level, as the $O_2$ concentration probably did not fluctuate very fast. 300 mya, atmospheric $O_2$ level was about 35%, much higher than today. To reach 35% $O_2$ as the $O_2$ Γ, $C_3$ plants must have ~700 ppm $CO_2$ (10). As the $CO_2$ decreased, the $O_2$ level must have also decreased to 21%. Decreasing $CO_2$ would have affected the $CO_2$ and $O_2$ competition for activities of Rubisco unless the $O_2$ also decreased to maintain a near constant ratio. $C_3$ plants cannot live in 300 ppm $CO_2$ and 35% $O_2$. This research area becomes a new topic for photosynthetic carbon metabolism, ecology, and geology to understand how the $CO_2$ and $O_2$ balance in the atmosphere is maintained by Rubisco.

As I drafted this prefatory chapter, I realized what a small part of this story (even work done by my associates) could be told in this limited length. It will take a lot of work to assemble all the previous papers from the past 80 years on $O_2$ and $CO_2$ effects on photosynthesis and plants and put them into perspective. In addition, the data are not all in, and different investigators will certainly continue to disagree on some points. Younger investigators can be excused for not understanding our past turmoil.

## *Eclectic Research*

There are constant temptations to spread one's research program too broadly. Such dilution has been dignified by claims of being a generalist in plant physiology. Our annual national meetings, textbooks, and Annual Reviews promote diversification. Many younger plant scientists do not have a focused plan in research but follow general areas and techniques. The most focused postdoc I had was W Outlaw, who would only work on guard-cell metabolism, although I knew nothing about it. I used much of my time and energy on many other topics besides those discussed in this prefatory chapter on the $C_2$ cycle. Those other associates were equally competent and appreciated. For the record, I have selected the reason and results of six of these other areas.

1. Research on phosphorylcholine in the 1950s and 1960s was started with H Wiebe under the general Atomic Energy Commission (AEC) theme of using radio isotopes for studies of metabolism. Only one $^{32}$P-labeled phosphate ester had a unique paper chromatographic RF of ~0.9 with 80% phenol-water (it was a Zwitter ion at the pH of phenol) but only ~0.15 with butanol-propionic acid (like other ionized phosphate esters). AA Benson's lab joined us in identifying it, and then B Martin, K Tanaka, and A Golke

studied its metabolism over 10 years. It is an immense, rapidly labeled pool (~20% of the total P) in plants moving in the xylem sap to the leaves, after formation in the roots. It is the only phosphate ester in plants that is transported. Much more needs to be done on its general role in regulating phosphate and lipid metabolism.

2. When searching for the importance of phosphorylcholine, I substituted a chlorine for the phosphate by synthesizing chlorocholine chloride, which was trivialized to CCC or Cycocel. CCC turned out to be an effective plant growth regulator for thick, short-stem wheat, rice, chrysanthemums, and poinsettias (work with S Wittwer). CCC was considered an antigibberellin after H Kende and colleagues found that it blocked GA synthesis, but alas, CCC had no apparent effect on phosphorylcholine metabolism. My bad guess led to a successful commercial product and I joined the Plant Growth Regulatory Society. You never know.
2. During my brief postdoc stay in Calvin's lab in 1950, P Pearson, Chief of the Biology Branch at the AEC, came to the lab looking for an assistant in plant physiology. Because most of Calvin's people were chemists with strong convictions about staying in California, it took but a moment for Calvin to decide that I should go to Washington, DC. That was a complete diversion from studying glycolate metabolism. At first I learned a lot from travel to projects in the USA, AEC labs, and Einewetok. Within two years, I wised up to the fact that giving out dollars was not going to get me much further in science. That was the first and last administrative job I ever took. I adopted the attitude that the perfect scientific environment is to be blessed with supportive, competent, leave-you-alone administrators. One such person, A Hollander, Director of Biology Division at AEC Oak Ridge National Lab, let me return to full-time research in plant physiology, meaning glycolate and sedoheptulose metabolism with P Zill, A Krall, and others. R Rabson, a postdoc with us, did valuable $^{14}C$ tracer work on the reactions of the glycolate pathway. He later proved me wrong about Washington, DC, by building a highly significant career supervising Department of Energy grants for research on plants.
4. When I was administrating plant physiology grants, S Hendricks, at USDA, Beltsville, and R Withrow, at Smithsonian, told me I was too young and rather stupid to have gotten out of research so early. Hendricks found me space and a technician in his lab, and I moonlighted studies on activation of glycolate oxidase in etiolated leaves with his spectrometer for whole plants. The action spectra was the same as photosynthesis—no big surprise even then. However, I was grateful for being kept scientifically alive. These two were experts on light action spectra for phytochrome and seed germination,

and their interest rubbed off on me, as indicated by latter publications with postdocs on seed germination.

5. After publications beginning in 1968 about leaf peroxisomes, I fancied myself a peroxisome biochemist. Michigan State University had established a medical school, and even though I avoided teaching medical students, NIH provided us funds for research on liver peroxisomes (6). Just as we all incorrectly thought that leaf peroxisomal metabolism was just a wasteful process, we also wondered whether peroxisomal respiration in animals, in contrast to mitochondrial respiration, might be controlling body weight. That grabbing hypothesis has not been supported by enough research to be accepted. Peroxisomes from liver, kidney, and elsewhere in the body have glycolate oxidase, and they oxidize long-chain fatty acids as do peroxisomes in germinating fatty seeds. How glycolate is formed in animals is not proven nor is its metabolic function understood. This research deviation from plants probably decreased our productivity in photosynthetic carbon metabolism, but striking similarities between leaf and liver peroxisomes augmented our peroxisomal studies with M Markwell, J Krahling, E McGroarty, R Donaldson, M Redinbaugh, S Furuta, J Uhlig, P Murphy, S Haas, S Vandor, and B Hsieh. Our lab and those of H Beevers and E Newcomb became places for research on plant peroxisomes. One of the most striking comparisons between plant and animal peroxisomes is how acetate from peroxisomal, long-chain fatty acid β oxidation is utilized. Animals form carnitine fatty acyl carrier complexes to move acetate and medium-length fatty acids from peroxisomes to other parts of the cell (work done with L Bieber's group). In the mitochondria, these carnitine derivatives are transferred to CoA and metabolized by the TCA cycle. Plant peroxisomes do not use carnitine as a carrier for fatty acids but rather completely degrade the fatty acid in their peroxisomes to acetyl CoA and condense it with glyoxylate to form malate. When this research on the glyoxylate cycle was initiated by H Beevers, he called peroxisomes by the term glyoxysomes. I followed de Duve's nomenclature for peroxisomes, which he originally discovered. In plants, this difference in nomenclature is not resolved and is confusing to others, so we have sometimes used "leaf peroxisomes" for the $C_2$ cycle. However, some peroxisomes are in all tissues of plants and animals.
6. I initially got interested in glycerol synthesis from the Great Barrier Reef expedition. Glycerol is an end product, just as is sucrose, of photosynthetic $CO_2$ fixation by symbiotic algae, zooxanthellae, which excrete it along with some glycolate to feed coral polyps. The triose phosphate shuttle between chloroplasts and cytosol transports the photosynthate, and there is just one

reduction step needed to form glycerol-P from dihydroxyacetone-P (DHAP). I was challenged by remarks from my contemporaries that there was no glycerol-P dehydrogenase in leaves. The secret, which R Gee and G Santora discovered for us, is that the plant enzyme is severely inhibited by trace amounts of any fats, lipids, and detergents in homogenates. A Goyal, RU Byerrum, T Kirsch, and D Gerber joined our project. There are major forms in the cytoplasm, in chloroplast, and in algae. They are very active as DHAP reductases for glycerol-P synthesis at pH 7.5, but nearly inactive as a glycerol-P dehydrogenase even at pH 9.5, so we call them DHAP reductases. A glycerol-P/glycerol metabolic cycle exists in algae for glycerol accumulation, and a longer-term goal would be to put this algal system in plants for stress protection.

## *Reviews, References*

With the proliferation of research, the number of references to a given topic becomes immense. A review seldom can include all references that are pertinent. An excuse to cite mainly your own is to say the number is restricted by the editor. Some particularly important paper(s) of your competitors need to be cited to show modesty and to appease in a grant application. The older literature is generally not cited because those authors are no longer around to review your paper or argue. In fact, by ignoring the older literature you can claim more originality (but also ignorance). Thus, older literature is never adequately covered, and your own work is never given enough credit.

I had thought that after retirement I would keep busy by writing reviews, but that was false thinking. In the first place, there is no inspiration to write reviews if you cannot experimentally test out the new ideas that float to the top. I am grateful to E Beck in Bayreuth, Germany, for doing our experiments on measuring the $O_2$ Γ, because otherwise I would be less motivated to write about nearly 80 years of work on $O_2$ inhibition of photosynthesis. Another issue is that it is horrifying how fast one becomes obsolete in research if you stop working on it. Keeping up requires attending meetings, scanning journals, and discussing research every day with active laboratory investigators. If one stops these activities for a year or even a few months, you are completely out, including the writing of reviews. Good reviews cannot be done in the absence of research. If you are a Distinguished Emeritus Professor, you might get away with memoirs without references about once, as in a prefatory chapter.

So then, what do you do with your office files? There is nothing so dear, yet so cold, as old lab notebooks, nothing so nostalgic as one's reprint and card file—but only to you. I have a collection since 1945 of most of the important

reprints on photosynthetic carbon metabolism, peroxisomes, and photorespiration, but it is of little value to others. However, I will hang on to it just as dearly as to life. With this tirade about references, I follow the editor's instructions to maybe cite a few, and, of course, they are all my own.

ACKNOWLEDGMENTS

This research occurred over a long period of time that was robbed from my family. I am most grateful to them for tolerating my single-minded devotion to it, which remains my only work and hobby. In the first draft of this chapter, I tried to mention names and results of all graduate students, postdocs, and visitors, who did the work. The review was far too long. For each of these associates our paths ran together for a valuable time, and I am forever grateful.

*Life hereafter continues in our genes; memory rests in our writings.*

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