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Minireview

Crassulacean acid metabolism photosynthesis: 'working the night shift'

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Abstract

Crassulacean acid metabolism (CAM) can be traced from Roman times through persons who noted a morning acid taste of some common house plants. From India in 1815, Benjamin-Heyne described a 'daily acid taste cycle' with some succulent garden plants. Recent work has shown that the nocturnally formed acid is decarboxylated during the day to become the CO_2 for photosynthesis. Thus, CAM photosynthesis extends over a 24-hour day using several daily interlocking cycles. To understand CAM photosynthesis, several landmark discoveries were made at the following times: daily reciprocal acid and carbohydrate cycles were found during 1870 to 1887; their precise identification, as malic acid and starch, and accurate quantification occurred from 1940 to 1954; diffusive gas resistance methods were introduced in the early 1960s that led to understanding the powerful stomatal control of daily gas exchanges; C_4 photosynthesis in two different types of cells was discovered from 1965 to ~1974 and the resultant information was used to elucidate the day and night portions of CAM photosynthesis in one cell; and exceptionally high internal green tissue CO_2 levels, 0.2 to 2.5%, upon the daytime decarboxylation of malic acid, were discovered in 1979. These discoveries then were combined with related information from C_3 and C_4 photosynthesis, carbon biochemistry, cellular anatomy, and ecological physiology. Therefore by ~1980, CAM photosynthesis finally was rigorously outlined. In a nutshell, 24-hour CAM occurs by phosphoenol pyruvate (PEP) carboxylase fixing $CO_2(HCO_3^-)$ over the night to form malic acid that is stored in plant cell vacuoles. While stomata are tightly closed the following day, malic acid is decarboxylated releasing CO_2 for C_3 photosynthesis via ribulose bisphosphate carboxylase oxygenase (Rubisco). The CO_2 acceptor, PEP, is formed via glycolysis at night from starch or other stored carbohydrates and after decarboxylation the three carbons are restored each day. In mid to late afternoon the stomata can open and mostly C_3 photosynthesis occurs until darkness. CAM photosynthesis can be both inducible and constitutive and is known in 33 families with an estimated 15 to 20 000 species. CAM plants express the most plastic and tenacious photosynthesis known in that they can switch photosynthesis pathways and they can live and conduct photosynthesis for years even in the virtual absence of external H_2O and CO_2, i.e., CAM tenaciously protects its photosynthesis from both H_2O and CO_2 stresses.

Abbreviations: CAM – Crassulacean acid metabolism; PEP – phosphoenol-pyruvate; Rubisco – ribulose 1,5-bisphosphate carboxylase oxygenase; C_3 – three carbon photosynthesis; C_4 – four carbon photosynthesis; PEP-Case – phosphoenol pyruvate carboxylase

Introduction

A host of plants with CAM photosynthesis live in the shadows of humanity without most people recognizing their unique daily activities. Worldwide in countless homes and workplaces CAM plants such as cacti, bromeliads, and *Sedums*, with many common names as jade, hen and chicks, Christmas cactus, and mother-in-law's tongue, live unobtrusively by their abilities to strongly control their use of H_2O and CO_2. Cactus lovers have even been immortalized in caricature as archetypal amateur naturalists, along with butterfly hunters and rock collectors, by the German romantic painter Carl Spitzweg (Figure 1). CAM plants can live, even indoors, with little attention for months and years; and then grow well and produce spectacular flowers when watered and given light. Few other higher plants can survive such extended neglect. The spectacular flowers of CAM succulents appear in early systematic botanical illustration collections, e.g., Basilius Besler's *Hortus Eystettensis* (1613) and *Codex Liechtenstein*. Of course CAM plants also occur naturally in abundance where they demonstrate powerful environmental survival abilities, e.g., desert cacti of southwest North America, the euphorbs of North Africa, bromeliads high in rainforest canopies, the *Aloe* 'trees' of the Kalahari Desert, or the 'spiny forests' of Madagascar. Also they are important crops, e.g., the plantations of pineapple in Hawaii and South America or *Agave* in Central America. Today CAM is known in 33 families of terrestrial and even in aquatic plants with an estimated 15 to 20 000 CAM species (Smith and Winter 1996; Winter and Smith 1996).

Indeed, because CAM plants often were present around humans, they were used in some historical pioneering research. For example, in Theodor de Saussure's respiration studies near 1804 he placed a cactus, *Opuntia*, in high (7%) CO_2 and observed both O_2 and CO_2 absorption in the dark. In contrast, other plants (with C_3 photosynthesis) absorbed only O_2. While he clarified the essential role of O_2 in respiration, the cactus use of both CO_2 and O_2 presented daily changes that remain unresolved even today. De Saussure did observe dark CO_2 uptake in a CAM plant, though this initial observation languished for about 1.5 centuries. In studies on 'Growth under Difficulties,' Charles Darwin asked if plants 'knew' which way to grow. For six months he hung a CAM plant, *Echeveria stolonifera*, by its detached stem upside down and observed the stem roots sprout and turn down and flowering branches bend upward, thereby helping to establish plant geotropism (Darwin 1877). While CAM or survival mechanisms were unknown to Darwin, tightly sealed stomata kept the unwatered detached plant quite viable. A century ago, researchers at the Desert Laboratory in Tucson, Arizona, documented the extraordinary water retention of CAM plants by periodically checking the small weight loss by an uprooted Saguaro, *Carnegiea gigantea*, that was suspended in the lab for several years. As we will see, CAM is a complex adaptation that allows photosynthesis to remain active in the virtual absence of external H_2O and CO_2 exchange, i.e., CAM controls and protects photosynthesis from H_2O and CO_2 stresses, certainly crucial photosynthesis traits in many environments.

CAM plants 'working the night shift'

How do CAM plants live phototrophically and so tenaciously achieve a degree of homeostasis seemingly independent of their environment? In a nutshell, one can say that CAM plants have devised ways to work and live each day on 'flex-time.' They have developed the ultimate separation between light dependent processes and some primary dark enzymatic reactions in photosynthetic carbon assimilation. The time frame of photosynthesis begins with light absorption near 10^{-15} seconds (Martin Kamen 1963) followed by reaction centers catalyzing oxidation-reductions near 10^{-12} seconds. But green tissues of CAM plants have 'stretched' oxygenic photosynthesis out to 24 hours by fixing CO_2 over a night period, i.e., 'working the night shift,' and then releasing that CO_2 during the day light period for photodependent reductive metabolism.

Knowing now that CAM comprises sets of daily interlocking reciprocal cycles, we can look back and analyze the long winding history of understanding CAM photosynthesis. Neimiah Grew highlighted an acid taste in *AIoe* in his 'An Idea of a Philosophical History of Plants' in 1682; indeed, it is claimed that an acid taste also was known to the Romans. In a letter to the Linnean Society in 1815, Benjamin Heyne described chewing and tasting the leaves of *Cotyledon catycine (Bryophyllum calycinum)* growing in gardens in India. After a night period, each morning the leaves were, 'as acid as sorrel' while other plant leaves were not; then the acid taste disappeared to a tasteless noon to a bitterish evening. Soon Link (1819) repeated Heyne's 'daily acid taste test' by measuring acid pH values with litmus paper. Thus for centuries a

Figure 1. Romantic paintings by Carl Spitzweg, 'cactus lovers,' illustrating the powerful environmental survival ability of CAM plants and human attachment.

'daily acid taste cycle' has been known showing that acids were formed at night and disappeared in the day in specific plants. In fact, Heyne's 'daily acid taste cycle' foreshadowed the direction of research even until today to understand CAM, namely, by measuring daily cycles! Even with these clues centuries would elapse before the acids and other components of daily CAM cycles would be positively identified, accurately measured, and CAM photosynthesis begin to be understood.

Origin of the acronym CAM

How did the term 'Crassulacean acid metabolism' originate? Clearly the initial work with some succulent plants in the family Crassulaceae was the origin along with the 'daily acid taste cycle.' Initially acid changes were strongly associated with tissue 'succulence'; but many succulent plants (e.g., halophytes) do not change acid levels daily. T.A. Bennet-Clark (1933a, 1949) used the phrases 'Crassulacean type of metabolism' and 'acid metabolism.' The German literature used 'diurnal acid rhythm' and J. Wolf even used 'Crassulacean malic acid' to describe a presumed isomer of malic acid (note that in 1942 G.W. Pucher and M. Nordal both identified it as isocitric acid, see Wolf 1937, 1960). The term 'Crassulacean acid metabolism' was first publically used in January 1947 by Meirion Thomas, in a Society of Experimental Biology talk and in the 4th edition of his textbook 'Plant Physiology'; formal publication was in 1949 (Thomas and Harry Beevers 1949). Subsequently, the easy acronym, CAM, has been widely accepted.

Landmark periods of creative ideas

Over the last two centuries, we note two great catalytic periods of creative progress in CAM photosynthesis research. The first, about 1875 to 1887, when the daily reciprocal relationships between acids and carbohydrates were observed experimentally. The second period, about 1960 to 1974, involved two critical discoveries. An immense stimulation came from the discovery of C_4 photosynthesis, which also catalyzed a revolution across all of plant biology (for a history of C_4 photosynthesis, see Hatch 2002). And almost simultaneously the critical roles of stomata in daily gas exchanges began to be elucidated with the development of gas diffusive resistance analysis methods.

Indeed these subjects continue to dominate thinking about CAM.

Daily reciprocal relationships during CAM

A landmark set of experiments were reported by Mayer (1875 to 1887) and Kraus (1884) who documented a daily reciprocal relationship between the total titratable acid and the carbohydrate (sugar) contents of green CAM tissues, generally indicating that carbon flowed back and forth between acids and carbohydrates each 24 hours. This work firmly set in motion the beginnings of metabolic understanding regarding CAM photosynthesis as a full 24-hour day. Mayer also found an anomalous type of photosynthesis, a light-dependent O_2 evolution in the absence of atmospheric CO_2exchange! The Mayer/Kraus studies on reciprocal cycles were very attractive and greatly stimulated later theories and studies because a host of ideas and experiments could be generated to explain the interactions of organic acids, carbohydrates, and environments over 24 hours.

Such landmark experiments were repeatedly confirmed, extended to other plants, and done with more environmental variations; particularly regarding irradiance duration and intensity, temperature, photoperiod, and various levels of O_2 and CO_2. Even so, understanding was limited for many years because precise identification of organic acids, carbohydrates, and the associated understanding of biochemistry and photosynthesis was absent. Also lacking were good procedures for extracting plants, separating enzymes and organelles, and techniques for identifying and assaying biochemicals. And importantly for green tissue photosynthesis, diffusion gas analysis techniques to understand stomatal functions were unknown. After World War I, several major groups (e.g., Wolf 1937, 1938, 1949, 1960; Bennet-Clark 1933a, 1949) worked extensively on CAM problems. While a few workers indicated other acids and carbohydrates (e.g., citric acid, pentosans or heptuloses) may exhibit small daily changes, the prevailing data showed that malic acid was the major acid and that starch was the major carbohydrate which changed during the daily reciprocal turnovers. Then, between 1919 and 1941, much crucially needed metabolic knowledge accrued in general biochemistry, i.e., enzymology, intermediary metabolism, and the tricarboxylic acid cycle, plus the presentation of a sound theoretical model for photosynthesis. After World War II, both quantitative and chromatographic techniques for measuring and identifying organic acids and carbohydrates became available. These developments lead workers to make accurate identifications and daily measurements regarding acids, carbohydrates, and the associated green tissue gas exchanges such as the respiratory quotient. Two active groups headed by H.B. Vickery and M. Thomas then provided some exceptionally strong data that supported later understandings of CAM photosynthesis.

Figure 2. A sketch of Hubert B. Vickery, Connecticut Agricultural Experiment Station, New Haven, USA. Dr Vickery (1893–1978). lived his career as a 'Chemist among plants' (Vickery 1972). His straight forward precise personality is quite evident when one evaluates the most exact values for the daily changes in acids and carbohydrates one can find in the CAM literature. He developed the analytical methods that finally (after 1.5 centuries) accurately identified and measured these daily biochemical cycles. With associates such as G.W. Pucher and C.S. Leavenworth he established the massive biochemistry that a plant can allocate to CAM; i.e., near 25 to 30% of a plants total dry weight may turnover each day in the reciprocal acid/carbohydrate cycles that supply photosynthesis with CO_2.

Hubert Vickery (Figure 2) and coworkers provided CAM research with its most precise analyses of organic acids and carbohydrates (Pucher et al. 1936, 1941, 1947; Pucher and Vicker 1942; Vickery and Pucher 1940). They identified all of the acids and accurately quantified the reciprocal daily changes in malic acid and starch/glucans under a variety of environmental conditions. They confirmed that CAM was

a very entrained rhythm, e.g., even when kept in constant darkness, a CAM plant would synthesize starch at the 'correct time-of-day.' Dark starch synthesis in green tissues was 'heresy'; everyone by then 'knew' that starch was synthesized during photosynthesis! So, under many imposed environments, the strong daily entrained nature of CAM became well known. Clanton Black once enticed a student, N.K. Chang, to measure CAM cycles in continuous darkness every two hours for a week. I can still 'see' his wife gliding into the lab with meals and 'hear' the alarm clock every two hours! In the imposed darkness, the plant continued its daily cycles, although at a reduced amplitude (Chang et al. 1981).

Meirion Thomas (Figure 3, photo) was fascinated by the O_2 and CO_2 exchange character of CAM plants and inspired a great group of students (see legend to Figure 3) to measure these gases and to calculate respiratory quotients (R.Q. = mol CO_2 evolved/mol O_2 absorbed) under a variety of experimental conditions. Thomas and his students had a clear, simple theory about the daily carbohydrate interconversions in CAM, in which they recognized that the classic Wood–Werkmann CO_2 fixation in nonphotosynthetic organisms (1938) could relate to dark acid CO_2 metabolism; they combined these with thinking about photosynthetic O_2 release and respiratory uptake. Their monumental set of studies showed that R.Q. values can vary from nil to 1.0 over a day (Ranson and Thomas 1960; Thomas and Beevers 1949). However, the associated biochemistry and stomatal activities were unknown; hence the net gas exchange values could not be understood. Nevertheless, Thomas inspired a remarkable group of students who subsequently made numerous original discoveries in plant biochemistry. For example, with CAM, David Walker found the primary carboxylase, PEP carboxylase, of dark CO_2 fixation (Walker 1956; Saltman et al. 1956) and an enigmatic asymmetric labeling of malate in the dark with $^{14}CO_2$ was soon noted (Bill Bradbeer et al. 1958).

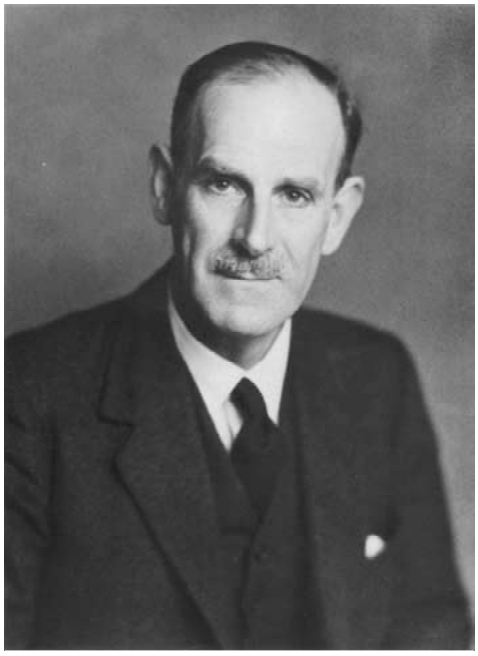

Figure 3. A photograph of Meirion Thomas, the University of Durham, Newcastle upon Tyne, England. Dr Thomas (1894–1977) known in his Welsh village as 'Thomas The Book' was a greatly respected teacher and researcher (Porter and Ranson 1980). His classic textbook, 'Plant Physiology' (in 5 editions 1935 to 1973) gave inspiration and direction to generations of British and colonial students of botany. He led one of the most notable groups of students ever assembled in plant research. That marvelous student group included J.W. Bradbeer, S.L. Ranson, H. Beevers, P.N. Avadhani, D.A. Walker, R.G. Paxton, R.F. Lyndon, and J.M.A. Brown. Knowing those lively students later in life, the authors imagine that the lab in Newcastle upon Tyne with M. Thomas was a sparkling, witty, fun place to be in the mid-20th century! They devised and performed perhaps the most ingenious and thorough sets of studies on CAM diurnal gas exchanges ever conducted. Their experiments to unravel the baffling daily changes in CAM plant respiratory quotients are classical accounts of how to approach solving a mystery. However, CAM plants were as tenacious about giving up their secrets about daily gas exchange and photosynthesis as they are in living well in some of nature's most stressful environments!

The critical role of stomata and gas diffusive resistances in daily CAM

Unfortunately, a fundamental trait of CAM plants was unknown for centuries, namely, the powerful control of gases by their stomata. Shortly after World War II, infrared gas analyzers were applied to CAM plants, and, for a short time, nocturnal CO_2 fixation in *Kalanchoe blossfeldiana* was even thought to be a flowering stimulus (Gregory et al. 1954). However, prior to the introduction of diffusion resistance analysis techniques to leaf photosynthesis research by P. Gaastra in 1959, the control of green tissue gas exchanges by stomata was hardly mentioned in CAM research. Although Nishida (1963) used other simple methods to show an 'inverted daily stomatal rhythm,' in which stomata were shown convincingly to open at night and close during some of the day, thus conserving water (Ekern 1965; Joshi et al. 1965), the significance of tight stomatal closure during the day in green CAM tissues for H_2O use efficiency or for photosynthesis

was not appreciated. Hence, those who studied CO_2 or O_2 exchange patterns could not know the extent to which stomata controlled the patterns of gas exchanges during CAM rhythms, e.g., under different O_2 levels (Moyse 1955) or in continuous light and dark regimes (Nuernbergk 1961; Warren and Wilkins 1961).

When infrared gas analysis was combined with gas diffusive resistance analyses, the surprises that followed measurements of stomatal conductance in CAM were quite astonishing. For example, the daily pattern of stomatal conductance closely followed the complex CO_2 fixation curve in Figure 4, i.e., stomata open at night and are tightly sealed during much of the day. Or even when wide open in the light or dark, stomatal conductances in CAM plants often are lower than those of closed stomata of C_3 plants in the dark. And recent experiments in air, at 380 μbar of CO_2, show that a combination of low stomatal conductance and low internal diffusion conductance, through tightly packed layers of green CAM cells, produces very low internal CO_2 concentrations, near 108 μbar of CO_2 (Maxwell et al. 1997). This tight closure of stomata during the most intense daily irradiances (phase III of Figure 4) clearly explained the efficient H_2O use during CAM photosynthesis (Black 1973). All diffusive resistance analyses with CAM tissues show the powerful control of gas exchanges by their stomata in each daily rhythm.

The daily CAM carbohydrate cycle

The daily reciprocal relationship of carbohydrates and acids was recognized near 1875 and generally starch was thought to be the major turnover carbohydrate. The 'closed loop' of a daily carbohydrate cycle can be a major metabolic investment, 25 to 30% of the total tissue weight can be in this day-night reciprocal cycle (see legend to Figure 2). Today we recognize that starch, smaller hexose polymers, and soluble sugar pools can furnish the three carbons required for PEP synthesis at night and are storage forms each day. A momentous push in understanding the daily CAM carbohydrate cycle came from understanding C_4 photosynthesis carbon biochemistry. 4-Carbon acid metabolism in CAM then could be divided into a night-time synthesis of PEP to form malic acid; a day-time 4-Carbon acid decarboxylation; and a return of the other three carbons (usually pyruvate) to storage pools. Hence, by 1972 one could postulate that carbohydrate metabolism was via portions of glycolysis at night plus portions of C_4 carbon photosynthesis during the day (Black 1973). But work on the actual pathway of daily reciprocal carbon flow in CAM was fairly quiescent, while work focused on PEP carboxylase and the decarboxylases for several years (Kluge and Ting 1978). This work clearly established that specific plants mainly used one of the three known decarboxylases; and PEPCase was noted as a key site of daily regulation. Since CAM photosynthesis biochemistry involved two competing carboxylases and a decarboxylase it was evident that untangling their control over 24 hours was paramount. Orlando Queiroz had found earlier (1967) that malate inhibited PEPCase activity, which Manfred Kluge recognized as a foundation to regulate PEPCase *in vivo* (Kluge and Osmond 1972). Even today, malate inhibition of PEPCase during CAM is an overriding component of one of the best documented regulatory cascades in photosynthetic metabolism (Hugh Nimmo 2000).

In an effort to quantitatively establish the reciprocal pathways of CAM carbohydrate metabolism, Nancy Carnal made the unexpected discovery of a very active pyrophosphate-dependent phosphofructokinase in pineapple leaves (Carnal and Black 1979). This discovery in CAM plants led the recognition of a new pathway of glycolysis in plants; led us to realize that pyrophosphate was a new and useful energy source in plants; led to the discovery of fructose 2,6-bisphosphate as our most potent regulator of plant carbohydrate metabolism; and led us to new aspects of sucrose metabolism (Carnal and Black 1983; Smyth and Black 1984; Black et al. 1987, 1995). These follow-up discoveries were an extraordinary outcome in plant biochemistry, gained from studying the daily carbohydrate metabolism of CAM photosynthesis!

C_4 photosynthesis and comprehending daily CAM

From about 1965 to the mid 1970s, a floodgate opened about new photosynthetic, biochemical, physiological, and anatomical information, particularly showing new roles for 4-carbon organic acids in photosynthesis. The insights into the biochemistry of CAM that flowed from the discovery of C_4 photosynthesis were already evident at a 1970 meeting in Canberra, Australia, where the spatial and temporal distinction between the carboxylation-decarboxylation events in the two pathways was widely accepted, especially when, with a

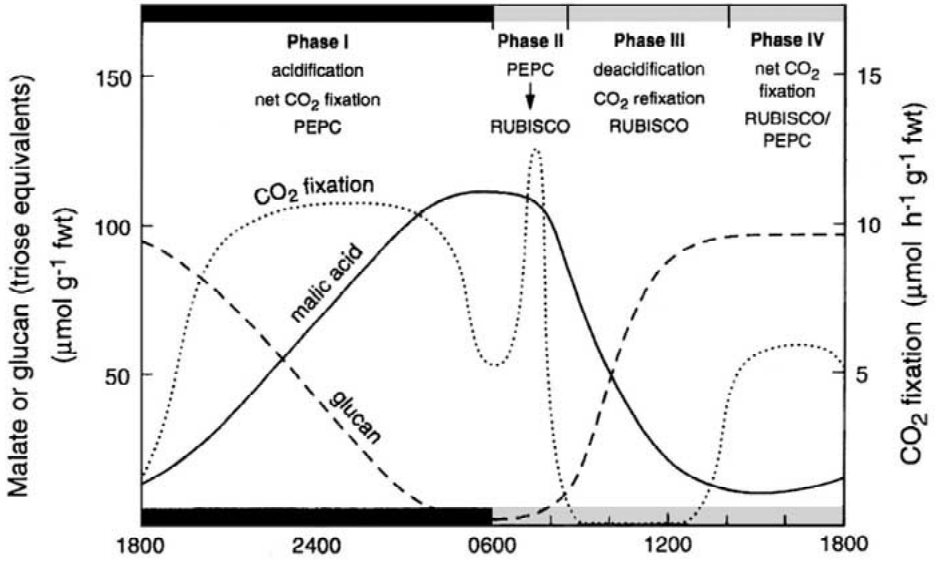

Figure 4. Typical daily patterns of CO_2 fixation and the reciprocal malic acid and glucan (carbohydrates) levels in a CAM plant given good cultural care, in full sunlight, with a 10 to 15 °C difference in the day vs. night temperature. To help understand CAM photosynthesis, the day is divided into four phases (Osmond 1976) with the main carboxylation and acid decarboxylation times noted. The exceptionally high internal CO_2 levels, 0.2 to 2.5%, noted by Cockurn et al. in 1979 occur in Phase III when the stomata are tightly closed. Note the closely coupled, but reciprocal, glucan (carbohydrate) and malic acid curves each day. PEPC – PEPCase.

deft twist, Mac Laetsch relegated the C_4 pathway to 'CAM mit Kranz' (Hatch et al. 1970). It was a quick, short step from learning in C_4 photosynthesis about a spatial, two cell, 4-carbon organic acid metabolism, to CAM photosynthesis with a temporal, two time-frame night and day, one cell, 4-carbon organic acid metabolism (Hatch et al. 1970; Black 1973; Burris and Black 1976; Osmond 1978; Kluge and Ting 1978). Even by 1972, a list of about 16 distinctive characteristics already defined CAM *versus* C_4 and C_3 photosynthesis plants (Black 1973).

Almost concurrently, the same enzyme activities were found in CAM and C_4 plants. But it quickly became clear that the 24-hour CAM photosynthesis was distinct from C_4. The temporal and spatial differences were obvious. Both formed malic acid through PEPCase and NADPH malic dehydrogenase. C_4 plants directed appreciable oxaloacetic acid to aspartic acid while CAM did not. The malic acid pool size in CAM were shown to be much larger and night malic acid storage was found in the vacuole (Kenyon et al. 1978). The quantative data of Hubert Vickery's lab (Figure 2) was recalled, showing the massive metabolic investment (25 to 30% of their dry wt.) by CAM plants. In specific C_4 plants three 4-carbon acid decarboxylases were found and similarly we found $NADP^+$- or NAD^+-dependent malic enzyme or PEP carboxykinase in specific CAM plants (Hatch et al. 1970; Dittrich et al. 1973; Dittrich 1975). The crucial enzyme to recover pyruvate carbon during the day and to form PEP for daily carbohydrate storage, pyruvate Pi-dikinase, was quickly found (Kluge and Osmond 1971; Sugiyama and Laetsch 1975). So, the enzymes required to conduct night C_4 acid synthesis, day decarboxylation, and portions of carbohydrate metabolism were present in CAM plants. Hence, the basic outline of a daytime, Rubisco-based, C_3 photosynthesis was soon established and integrated with the supporting daily reciprocal malic acid and starch (carbohydrate) cycles (Figure 4).

As the remarkable flow of new information from C_4 photosynthesis studies spilled over into quickly learning how CAM photosynthesis functioned, another strong group of people were drawn into CAM work including Manfred Kluge, Irwin Ting, Orlando Queiroz, Ulrich Lüttge, Marie L. Champigny, T.F. Neales, T. Sugiyama, Mac Laetsch, Margaret Bender and the authors. And this research vortex continued on with other eras of CAM students, e.g., Klaus Winter, D.J. von Willert, Marion O'Leary, Joe Holtum, Stan Szarek, Deborah Sipes, B.G. Sutton, Iraj Rouhani, Ali Moradshahi, Park Nobel, J.C. Lerman, Z. Hanscom, M.B. Jones, Clif Crews, Craig Martin, Peter Dittrich, Bill Cockburn, Martin Spalding, Lonnie Guralnick,

Bill Kenyon, Nancy Carnal, J.A.C. Smith, Ernest Medina, and E.D. Schulze, C.S. Hew, E. Ball, John Nishio, Ray Chollet, Bill Allaway, Hugh Nimmo, Elizabeth Olivares, Annie Borland, Howard Griffiths, John Cushman and others today.

CAM photosynthesis

Suddenly within a decade, ~1968–1980, a rigorous mechanistic outline of daily, 24-hour, CAM photosynthesis became possible! During this time period, this 'critical mass' of workers integrated the previous 1.5 centuries of somewhat inexplicable CAM experiments, by using three separate lines of research, to finally elucidate the major aspects of CAM photosynthesis. First, the rapid knowledge accumulation about 4-carbon acid biochemistry combined with intermediary carbohydrate metabolism knowledge gave a sound foundation for understanding the internal 'black box' of CAM tissue starch/glucan and organic acid metabolism. Second, the ability to continuously measure CO_2 and O_2 exchange, stomatal resistance, and transpiration over 24 hours gave a very revealing picture of whole tissue CAM photosynthesis that could be compared with C_3 and C_4 photosynthesis.

Third, the exhilarating discovery in 1979 of highly elevated internal green tissue gas levels cemented the total integration of these research avenues into a rigorous mechanism for CAM as a '24-hour photosynthesis.' Bill Cockburn et al. (1979) discovered that when CAM plant stomata close tightly in the light in response to the internal generation of CO_2 (by the decarboxylation of malic acid, Figure 4), this results in the generation of very high CO_2 concentrations, up to 25,000 μbar of CO_2 internally (Cockburn et al. 1979), in photosynthetic tissues, with a simultaneous accumulation of high O_2 concentrations, up to 41.5% (Spalding et al. 1979). The Cockburn et al. studies showed that various CAM plants elevate their internal CO_2 from 0.2 to 2.5% during malic acid decarboxylation (phase III, Figure 4). It was immediately evident that this CAM CO_2 concentrating mechanism could very effectively reduce photorespiratory carbon cycling, even with an elevated O_2; in fact the carboxylase/oxygenase ratios for Rubisco under these extraordinary conditions have been estimated at 2.5 to 11 times larger than those of spinach or tobacco in air (Osmond et al. 1999).

It is intriguing to note that Stanely Ranson was the thesis advisor of Bill Cockburn. Hence Bill is a 'scientific grandchild' of Meiron Thomas; and perhaps he was predestined to furnish the internal gas values for CAM tissue which help explain their external gas exchange values, e.g., the inexplicable daily changes in R.Q. values, measured in the Thomas laboratory three decades earlier.

By 1980, the convergence of these three research lines revealed how 24-hour CAM photosynthesis (Figure 4) functioned in following the sequence of events:

1. A nighttime formation of PEP from stored carbohydrates; nocturnal stomata opening; PEP carboxylation (with HCO_3^-) followed by malic acid synthesis; and then malic acid storage in vacuoles.
2. A shift in the early dawn in CO_2 fixation with stomata blinking open then closing tightly; followed by malic acid decarboxylation with the internal green tissue CO_2 concentrations rising to ranges from 0.2 to 2.5% to highly favor Rubisco; C_3 photosynthesis through Rubisco operating during most of the day to fix CO_2 and to synthesize carbohydrates including the recovery and storage of the 3 carbons from decarboxylation, i.e., usually pyruvate; and little H_2O loss during the maximum day irradiances due to tightly closed stomata (phase III, Figure 4).
3. Late in the afternoon stomata may open and lesser amounts of CO_2 can be fixed (Phase IV). The amounts of late afternoon CO_2 fixation is quite variable, zero to ~40%, in literature reports.

In the literature, there are many variations on this typical photosynthesis outline (in Figure 4) because CAM is so responsive to prevailing environmental conditions. For example, many workers have varied irradiance intensities, temperatures, photoperiods, O_2 and CO_2 levels, plant development, salinity, or water levels. All have influences on CAM photosynthesis over 24 hours. The amplitude of daily acid change and the amounts of day *versus* night CO_2 fixation are very sensitive to environmental changes. However, these remarkable plants endure these changes and may live for months and years with such environmental stresses, including total detachment from soil and H_2O, likely because they have such a powerful tenacious control of their CO_2 and H_2O supply (both required for oxygenic photosynthesis).

Switching photosynthesis pathways between CAM, C_3, and C_4

In the 1970s, the adaptability of CAM, both as a constitutive and inducible photosynthesis, became evident to many researchers (Kluge and Ting 1978). Entirely unexpected variations in CAM photosynthesis were discovered in 1972–1973 as we were in the midst of great excitement about applying new 4-carbon biochemistry and other new findings to CAM. We knew that carbon isotope discrimination studies were showing that C_3 plants discriminated against ^{13}C at Rubisco and that C_4 plants with PEPCase (which fixed HCO_3^-) did not strongly discriminate against ^{13}C. Indeed, carbon isotope discrimination values, $\delta^{13}C$, cleanly separated C_3 and C_4 plants into two groups (Bender 1968; Smith and Epstein 1971). Then, surprising carbon isotope evidence began to accumulate showing that CAM plants might shift between their dominant primary carboxylases, hence shift their major pathway of photosynthesis. To these authors, one of the highlights in our CAM studies occurred aboard the RV *Alpha Helix* during the Great Barrier Reef Photorespiration Expedition in 1973. The setting, and the liberal exchange of views promoted by Ed Tolbert, Bob Burris and Andy Benson, led to many ideas which our competing labs quickly pursued. In Athens, Georgia, Iraj Rouhani had contacted Margaret Bender in Madison, Wisconsin. They had collected data using isogenic clones of known CAM plants showing $\delta^{13}C$ values from -14 to –32‰, which ranged over both C_3 and C_4values (Rouhani 1972). Barry Osmond indicated they had similar $\delta^{13}C$ results in Australia. On the Reef, we immediately recognized in our results the now obvious contributions of C_3 and C_4 carboxylation reactions to the variable $\delta^{13}C$ values of CAM plants and quickly submitted our sets of CAM $\delta^{13}C$ work for publication (Bender et al. 1973-received May 30, published November; Osmond et al. 1973-received April 26, revised June 5, published November). Hence, in an uncommon ability, amongst plants, CAM plants can switch their primary photosynthesis between CAM and C_3 photosynthesis depending upon their environment!

Those observations were readily repeatable in controlled environments (Burris and Black, 1976). Barry Osmond was able to drive the $\delta^{13}C$ values of *Kalanchoe diagemontiana* from –30 to –18‰ by manipulating light intensity, temperature and water stress in the cross-gradients of the University of Wisconsin-Madison Biotron; and importantly Barry stimulated Marion O'Leary to convert our then muddled thinking on the isotope discrimination process into a plausible framework. Indeed, Marion first delineated the diffusional and biochemical components of the $\delta^{13}C$ signal by examination of the $\delta^{13}C$ value of the 4-C carboxyl of malic acid in CAM plants (O'Leary and Osmond 1980); and set the stage for the now widely used $\delta^{13}C$ analysis of water use efficiency in C_3 photosynthesis. Little did anyone imagine that these CAM insights would ultimately lead to the selection of more water use efficient wheat and other crops now worth hundreds of millions of dollars annually in Australia alone.

During the early $\delta^{13}C$ studies, it became clear that CAM photosynthesis was both inducible and constitutive. Numerous plants express CAM throughout their autotrophic lifetime. But another difficult problem in CAM photosynthesis is that, with a few plants, the stage of plant development affects CAM expression in green tissues, in combination with environmental stresses. For example, in nature *Mesembryanthenum crystallinum* will express C_3 photosynthesis when young and slowly shift to CAM as it matures and is water stressed. A unique and exciting experiment was reported in 1972 by Klaus Winter and Dieter von Willert who discovered that NaCl induced CAM photosynthesis in *Mesembryanthemum crystallinum* when the plant was conducting C_3 photosynthesis. Klaus went on to more fully explore the ecological, physiological, biochemical and regulatory implications of this remarkable C_3 to CAM transition induced by NaCl (Winter et al. 1978; Winter and Smith, 1996). Since salt treatment is easily monitored, this transition has become the model even today for molecular work on the induction of 4 carbon acid activity. Thus, we learned that two well-documented environmental stresses, excess salinity or extreme drought, can induce CAM in a variety of plants. For example, *Portulacaria afra* expresses mostly C_3 photosynthesis under good cultural environments, but under H_2O stress it shifts to CAM photosynthesis (Ting and Hanscom 1977). Some *Kalanchoe* species have similar shifts with H_2O or NaCl stress (Kluge and Ting 1978) and some CAM species, e.g., *Agave deserti*, when grown under dry conditions, will switch to C_3 photosynthesis when irrigated (Hartsock and Nobel 1976).

A switch from C_4 to CAM photosynthesis was noted by Clanton Black who observed a vital result (see Figure 5 legend) for *Portulaca oleoracea* when it faced a fatal situation of uprooting by farmers and drying in the tropical sun. Leaf C_4 photosynthesis stopped

Figure 5. Photograph of an ecological physiology excursion of Clanton Black to San Ramon, Peru, in 1982, where he chanced on a pineapple, *Ananas comosus,* plantation in full competition with the C_4 crabgrass *Digitaria sanguinalis*. The competitive nature of C_4 plants (crabgrass) had been well known to him since his farm youth (Black et al. 1969), but the competitive nature of a CAM crop (pineapple) was a surprise. Orchids, the swaying whisker-like 'grey moss,' and other bromeliads clinging to trees hardly appeared to be competitive plants on our Florida farm! Equally unexpected to Clanton was to find that CAM was expressed by a known C_4 plant, *Portulaca oleoracea*, when Peruvian farmers pulled and turned it uprooted to dry in the tropical sun. In that seemingly fatal uprooted and unwatered situation this highly competitive weedy plant (Black et al. 1969) expressed CAM in its green stems and branches, thereby using CAM photosynthesis to support an abundant seed production for its next weed growing season as a C_4 plant! This was an 'unlooked for' switch from C_4 to CAM photosynthesis that overcame uprooting by farmers, apparently for the normally weedy C_4 plant to reproduce!

and CAM was expressed in stems and branches. This was followed by *P. oleracea* flowering and abundantly producing seeds. Clearly, with this competitive weed, CAM photosynthesis is an adaptation to support reproduction (and to infest crops the next season)!

Because of these abilities to shift their pathway of photosynthesis, we consider CAM-expressing organisms as our most plastic oxygenic photosynthetic organisms! As noted, the expression of CAM is under both developmental control and environmental influences, i.e., CAM is both constitutive and inducible in given species. Indeed when switching between CAM, C_3 and C_4 photosynthesis under various environments and in various species, one can ask, 'When is a plant CAM'? CAM is a somewhat arbitrary designation since a given plant can range from nil to near 80% (as in Figure 4) and even up to 100% of its CO_2 fixation at night. Or have no net CO_2 fixation when uprooted or detached; and yet conduct daily CAM photosynthesis internally in this extreme condition. Although no clear cut answer exists, historically two key CAM traits are night CO_2 fixation and efficient H_2O conservation, both related to supplying CAM photosynthesis with two essential components, CO_2 and H_2O!

CAM photosynthesis prospectus

Certainly with so many land and water CAM species (perhaps 20,000), such strong environmental control traits, and being both constitutive and inducible, CAM remains with many useful and informative facets to be discovered! For example, two new types of photosynthesis have been discovered recently which portray types of photosynthesis that are intermediate between CAM and C_4. Within single cell marine diatoms the light dependent pathway for furnishing CO_2 to Rubisco apparently is: salt water $HCO_3^- \rightarrow$ PEP carboxylase $\rightarrow$ C_4 organic acids $\rightarrow$ decarboxylation $\rightarrow$ $CO_2 \rightarrow$ Rubisco $\rightarrow$ C_3 photosynthesis (Reinfelder et al. 2000). Theoretically in the ocean CO_2 limits Rubisco; but with an excess of HCO_3^-, C_4 acids can be produced via PEPCase; hence the ocean HCO_3^- can furnish photosynthesis with ample CO_2. With the desert plants, *Borszczowia aralocaspica* and *Bienertia cycloptera,* growing about the Aral Sea in Central Asia and southward to the Indian Ocean, a day time only fixation of CO_2 occurs in a single green cell type by a pathway similar to CAM (Voznesenskaya et al. 2001; Freitag and Stichler 2002). In deserts, H_2O limits plants. Therefore, through C_4 acid formation and decarboxylation in one green cell, these organisms apparently capitalize on two strong adaptative CAM traits, namely, protecting photosynthesis from H_2O and CO_2 stress, i.e., protection against a CO_2 limitation in marine diatoms and a H_2O limitation in desert plants. To paraphrase Mac Laetsch, these organisms express 'CAM without darkness.'

Finally, perhaps inspired by Heynes field observations of a 'daily acid taste cycle,' many CAM researchers have conducted field expeditions to evaluate CAM from various perspectives such as acidity or its remarkable H_2O and CO_2 conservation abilities under natural conditions. Such enterprises led Clanton to South America (Figure 5) and recently to Central Asia, Mongolia and Tibet; Barry (Figure 6) into fieldwork in the European Alps (Osmond et al 1976) and to several excursions seeking to discover how prickly pear came to be such a successful weedy pest in eastern Australia (Osmond and Monro 1981); Park Nobel to Mexico and Central America with *Agave* and *Opuntia*; Ulrich Lüttge to Central and South America

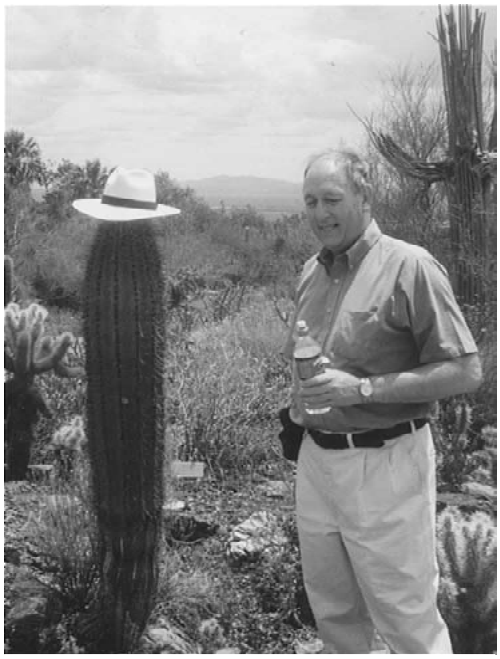

Figure 6. 'Hats off to CAM.' A photograph of Barry Osmond as a CAMpaigner in 'cactus heaven.' Barry now finds ample opportunities to examine the effects of excess light and water stress on photosynthesis in Sonoran Desert succulents. Among other things, Nobel and Bobich (2002) used the 300 m^3 'Klugekammer' closed gas system at the Biosphere 2 laboratory to show that all of the carbon fixed in the dark by droughted CAM succulents after irrigation, and 6-fold more carbon from reserves, is directed to root growth. Photo courtesy of Dr Britta Förster.

with epiphytes and *Clusia*; Howard Griffiths to South America with *Tillandsia*; Jon Keeley to the ocean with aquatic CAM; and Klaus Winter even moved to Central America, Panama, to work with tropical CAM species. Evidently, CAM is the attractive personification of photosynthetic metabolism for every 24 hours and for all seasons!

Concluding remarks

In trawling through centuries of CAM history, we hope the incisive ideas and events were properly hooked. Understandably, in this history of CAM photosynthesis, numerous intriguing and important aspects of how CAM functions are not presented, such as daily rhythms or clocks; the cellular flow of metabolites between the cytoplasm, vacuoles, mitochondria, and chloroplasts; membrane transport in and out daily; the daily allocation of resources from green tissues to heterotrophic plant growth; the biochemical and photosynthetic heterogenicity of green CAM tissues; regulation of daily cycles at many levels from gene expression to whole plants; environmental signal transduction; or CAM energy costs. Indeed, important variations in CAM biochemistry in specific plants and in environmental adjustments are worthy of separate presentations, such as how CAM functions without stomata in aquatic and rain forest environments or the citric acid metabolism of *Clusia*. No researcher should feel slighted because we focused on photosynthesis ideas and events rather than separate experimental results, species, or other aspects of CAM. In fact, today, many excellent detailed experiments support this centralized account of CAM photosynthesis and other daily CAM functions.

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