Review

Crassulacean Acid Metabolism: Now and Then

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Previous title chapters in Progress in Botany, from giants of European botanical research in the latter half of the twentieth century, have explored significant areas of plant science. I am honoured, and more than a little over-awed, by the Editor's invitation to contribute in this context (and alarmed to discover that I am only, but precisely, a decade younger than the previous contributor!!). Although this chapter may not present the long view of the discipline offered by others, any perspective on crassulacean acid metabolism (CAM), a pathway of photosynthetic carbon metabolism that occurs in about 5% of vascular plants (Winter and Smith 1996; Lüttge 2004), reveals much of wider significance in plant physiology and biochemistry. When Clanton Black and I prepared a brief historical overview of CAM (Black and Osmond 2003), we emphasized the close relationship between these succulent plants and humans through the romantic paintings of Carl Spitzweg. We noted that the taste–test diagnostic of CAM may have been known to the Romans, and that accounts of morning acidity in leaves of succulents that disappeared by evening were published by Grew in the seventeenth century and Heyne in the early nineteenth century. The early literature on CAM into the 1960s was highlighted, but space constraints relegated most of the influential studies of the last 40 years to little more than a few citations from more than 40 mentors, former students and colleagues.

This chapter provides an opportunity to make amends by recording my indebtedness to very many companions in CAM research. It is a personal view of an active and exciting area of plant biology since about 1970. Indeed, Lüttge (2004) cited a selection of more than 20 reviews, edited volumes and books on CAM (notably Kluge and Ting 1978; Winter and Smith 1996) over the last 2–3 decades. My reminiscences will be largely confined to areas in which my companions and I have published, but the temptation to range more broadly sometimes will be difficult to resist. Throughout, I will link to important current developments, and emphasize some broader implications that have emerged. As will become evident, my peripatetic research on photosynthesis in succulent plants with CAM continues to depend on stimulus from many colleagues in plant science, particularly those in Germany.

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1.1 A pathway to CAM via oxalate and malate in *Atriplex*

There was little in my family or educational background to suggest any particular scholastic ability or affinity with plant biology. Alfred Kurtz, a distant relative of my mother, and a well known second generation viticulturist in the Mudgee region of central western NSW, was the only strong family connection to things botanical. His vineyard is generally credited as the source (in the 1950s) of robust Chardonnay root stocks that supported the worldwide expansion of this variety in the last half of the twentieth century (Halliday 1985). However, since both sides of my family were teetotal for two generations, I did not think to explore viticulture or enology. Rather, my botanical career emerged accidentally. It was made more likely by a spectacular collapse in mathematical ability between high school and university that terminated my aspiration to qualify as a teacher of math and science. After 3 undistinguished years, I began again, and in 1960 did well enough in botany and in natural products organic chemistry to commence graduate research in botany at the University of New England, Armidale NSW, then the only university in Australia outside a State capital city.

I was influenced by the ecological focus of the Armidale Botany Department especially its interest in halophytes of the genus *Atriplex*, the "saltbushes" from semi-arid ecosystems in southern Australia. Physiological plant anatomy featured in the curriculum, and *Atriplex* leaves were fascinating for their large crystals of calcium oxalate, their huge epidermal bladders that proved to be salt secreting systems, and their "Kranz" arrangement of mesophyll and bundle sheath tissues that proved to be the foundation of C_4 pathway of photosynthetic metabolism. One could not have been presented with a more fascinating complex of leaf physiological anatomy, and all three features were to provide this starting graduate student with significant opportunities for original research. My first encounter with a CAM plant in the wild (a huge specimen of "tree pear" introduced O*puntia tomentosa* Salm-Dyck) took place about 1960 during an excursion to the arid shrublands of south west Queensland as a field assistant to Professor Noel Beadle, a pioneering Australian plant ecophysiologist. Sadly, it was a decade or more before I rediscovered the impact of CAM on the Australian landscape.

Presented with equipment for ether extraction of organic acids, I found oxalate to be the balancing anion for the inorganic cation excess in *Atriplex* leaves. Subsequently, as a PhD student in the laboratory of Professor "Bob" Robertson in the University of Adelaide, it was possible to explore the synthesis of oxalate following $^{14}CO_2$ fixation in the light and dark, using ion-exchange and paper chromatography. Malic acid, exclusively labelled in

the 4-C carboxyl was the most abundant early labelled product in the dark (the terms malic acid and malate will be used interchangeably throughout this chapter). Unexpectedly, 4-C labelled malic and aspartic acids, were also the most abundant initial products of $^{14}CO_2$ fixation in the light in *Atriplex* leaves. As related elsewhere (Osmond 1997), I had stumbled across the "β-carboxylation" pathway of primary CO_2 fixation, subsequently associated with "Kranz" anatomy, in this large genus of C_4 plants.

These early adventures in intermediary metabolism were stimulated by P.N. ("Danny") Avadhani, who was visiting Adelaide from the University of Singapore. Danny considered himself an "ideas man" and he occupied the chalkboard in the Departmental tearoom for days on end with a frequently amended forerunner of the metabolic wall charts that Boehringer-Mannheim later supplied to decorate laboratories throughout the world. Our interpretation then of the pathway of oxalate synthesis in *Atriplex* leaves (Osmond and Avadhani 1968) was based on analogies with the isocitrate cycle and was probably incorrect. Danny had taken his PhD in the University of Newcastle upon Tyne, where Thomas and Beevers (1949) had introduced the term Crassulacean acid metabolism. In the same laboratory, David Walker (1956) had demonstrated that phosphoenolpyruvate carboxylase (PEPCase), the legendary "wouldn't work man!" reaction (Wood and Werkmann 1938), was involved in the pathway to malic acid in CAM. It is a particular pleasure now to observe that another generation of researchers has been "taking coal to Newcastle" in the form of highly original physiological biochemical and unequivocal molecular evaluations of CAM (Griffiths et al. 1989; Borland et al. 1999; Borland and Dodd 2002).

I had been introduced to plant physiology through the 1956 edition of Thomas' textbook but its description of CAM in terms of respiratory quotients probably explains why I did not readily connect to the photosynthetic implications of this pathway. Even now, with O_2- and CO_2-specific electrodes (Osmond et al. 1996) and mass spectrometers (Maxwell et al. 1998), the stoichiometries of net CO_2 and O_2 exchanges in CAM are difficult to interpret. Ranson and Thomas (1960) provided the authoritative source on CAM in English, but it had been reported that malic acid accumulating in CAM in the dark was labelled in both 1-C and 4-C carboxyl positions, in the ratio 1:2 (Bradbeer et al. 1958). At the time, the "Newcastle overall hypothesis" seemed rather perplexing and only remotely connected to my observations in *Atriplex*. Nevertheless, Danny led me through the simple diel routine of acid extraction in boiling water and titration to phenolphthalein end-points that even now draws me through nights of interrupted sleep. There is no escape from this fundamental reference for the temporal expression of CAM in different conditions, a simple reference that could

bring greater rigor to contemporary studies of regulatory cascades of gene expression.

Evidently a slow learner, I should have recognized the research potential of CAM during a postdoctoral year in George Laties' laboratory at UC Los Angeles exploring ion transport and malate compartmentation in beet discs (Osmond and Laties 1967). Ulrich Lüttge occupied the other side of the lab bench, and although I doubt we spoke of it then, our lifelong friendship later came to be entwined with CAM. A second post-doc year with Tom ap Rees in Cambridge introduced me to enzymology and the use of specifically labelled substrates for evaluation of metabolic pathways. I then had the good fortune to join Ralph Slatyer's Department of Environmental Biology in the new Research School of Biological Sciences (RSBS) at the Australian National University (ANU) in Canberra, and returned to Australia in 1967. The "research only" appointments in the Max Planck-like Research Schools embedded in a university environment, with limited opportunities for tenure but access to front-line equipment of the day, provided privileged starts for many research careers in Australia at the time. I had been hired to work on starch to malate metabolism in stomatal guard cells, but any links to CAM research that may have occurred to me at the time were soon put to one side by the wave of interest in C_4 photosynthesis. The Research School was just across the street from CSIRO Plant Industry, the nation's strongest concentration of plant physiologists and biochemists, notably Jan Anderson, Keith Boardman and Hal Hatch.

Ralph's prestige, and the popularity of environmental science at the time, may have conspired to grant us the opportunity to organize the first workshop sponsored by the US–Australia bilateral programme in science and technology. With Ralph's deft handling, the programme was expanded to include some leading scientists from the UK, Germany and Japan. The ensuing workshop on photosynthesis and photorespiration was most timely and evidently of lasting impact (Sage and Monson 1999). It was a very exciting time in photosynthetic metabolism, and the meeting afforded excellent early career opportunities to build enduring networks. My latent interest in CAM was stimulated by this meeting when a plant anatomist (Laetsch 1970) provocatively declared the C_4 pathway to be "CAM mit Krantz". A better understanding of CAM in relation to C_3 and C_4 pathways of metabolism was obviously needed, especially after the surprising observation of Klaus Winter that the ice-plant *Mesembryanthemum crystallinum* L. could be converted from C_3 to CAM patterns of CO_2 fixation by salt stress (Winter and von Willert 1972). Hal Hatch was providing inspirational research leadership in C_4 metabolism in CSIRO, and the time seemed ripe to explore CAM as a photosynthetic process.

1.2 Sorting the phases of CAM

We now know that C_4 and CAM pathways of photosynthetic carbon metabolism are both based on largely analogous preliminary CO_2 concentrating mechanisms (CCMs) in which primary carboxylation leads to 4C acids (and amino acids) that serve as intermediate, internal stores of carbon. These substrates are subsequently decarboxylated to generate internal CO_2 concentrations of 1000–25,000 ppm (Cockburn et al. 1979) that largely mitigate the oxygenase activity of Rubisco (Leegood et al. 1997). From an evolutionary perspective, these CCMs recreate the atmospheric CO_2 concentrations of the Cretaceous, a time of grand expansion of terrestrial plants under conditions of CO_2 saturation in which O_2 fixation by Rubisco oxygenase and subsequent C recycling in photorespiration would not have carried the same penalty, in energetic terms, as it does for C_3 photosynthesis today. Simply put, CCMs of C_4 plants are based on small (about 1–10 mM), spatially separated cytoplasmic pools of 4C acids that turn over rapidly ($t_{1/2}$ about 1–10 s). These CCMs can be distinguished from those of CAM plants which are based on larger (100–500 mM) pools of 4C (and 6C) acids in the vacuoles, that turn over much more slowly ($t_{1/2}$ about 5000–50,000 s) with complex, temporally separated, patterns of acid synthesis and degradation.

With a lot of help from colleagues, I set out to impose some order on the carbon metabolism of these temporally separated processes (so-called phases I–IV) and to place the curiosity of CAM into the context of other pathways of photosynthetic metabolism. The 1970 workshop stimulated two CAM enthusiasts, Manfred Kluge and Irwin Ting to spent sabbatical periods in RSBS where they successfully demonstrated the presence of pyruvate Pi dikinase and the distinctive kinetic properties of PEPCase in extracts of these plants (see below). Bruce Sutton, my first PhD student, undertook a reassessment of the labelling patterns of malic acid in CAM plants exposed to $^{14}CO_2$ in the light by comparing the previously employed *Lactobacillus* culture degradation method and degradation with purified malic enzyme to remove the 4-C carboxyl of specifically labelled malic acid preparations (Sutton and Osmond 1972). These experiments strongly suggested that fumarase activity in *Lactobacillus arabinosus* (synonym for *L. plantarum* WCFS1) led to randomization of label from 4-C to 1-C in ^{14}C-malic acid prior to or during decarboxylation, especially when old cultures were used to degrade large amounts of malic acid. Indeed, using the purified enzyme, we found that malic acid from dark $^{14}CO_2$ fixation in CAM plants was initially and predominantly 4-C labelled, consistent with primary CO_2 fixation of unlabelled PEP by PEPCase. On the other hand, malate labelling in the light was closer to the 1-C to 4-C ratio of 1:2 observed by Bradbeer et al. (1958),

consistent with PEP formation from two molecules of PGA, one of which had been previously labelled as a result of prior $^{14}CO_2$ fixation by Rubisco (Osmond and Allaway 1974).

Generous sabbatical provisions in ANU (1 year in 4 for tenured staff; a legacy of the postwar sense of isolation down-under) enabled me to work in UC Santa Cruz and the Technische Universität, München in 1973–1974. Harry Beevers evidently had a soft spot for CAM from his days in Newcastle upon Tyne and was a most generous host in Santa Cruz. Although germinating castor beans were an ideal system for investigation of Rubisco in proplastids, like most others in the lab, I welcomed opportunities to escape the nauseous extraction process. It proved possible to commute over the coast range for nocturnal gas exchange experiments with CAM plants in Olle Björkman's lab at Carnegie Plant Biology, Palo Alto. By the time I joined Professor Hubert Ziegler in München, it was clear that CO_2 fixation in the dark in CAM plants was insensitive to O_2, whereas CO_2 fixation in the light was inhibited by O_2 (Björkman and Osmond 1974), further confirming that C_4- and C_3-like carboxylation systems were functioning in a temporally separated fashion.

I went to München because Professor Ziegler had excellent access to natural abundance ratio mass spectrometers. We and others had earlier speculated that the variable natural abundance $\delta^{13}C$ values of CAM plants might reflect the variable contributions of C_4- and C_3-like carboxylations in the dark and light (Bender et al. 1973; Osmond et al. 1973). With time to think and colleagues to challenge, it now seems natural that notions of the "phases of CAM" should have matured in München, to emerge then in Naturwissenschftliche Rundschau (Osmond and Ziegler 1975; Fig. 1), some time before their most commonly cited source (Osmond 1978). Much more comprehensive studies have subsequently refined the above simple interpretation of $\delta^{13}C$ values in different taxa in different environments (Winter and Holtum 2002; Holtum et al. 2005a).

Unequivocal and independent confirmation of the labeling patterns in Fig. 1 followed later from gas chromatograph-mass spectrometry (GCMS) analysis of ^{13}C-malate extracted from CAM plants after exposure to $^{13}CO_2$. Only singly labelled malic acid molecules were detected in the dark (Cockburn and MacAuley 1975), with doubly and multiply labelled molecules appearing during $^{13}CO_2$ fixation in the light (Ritz et al. 1986; Osmond et al. 1988). Griffiths et al. (1990) provided the ultimate proof of the shifting carboxylation activities in the phases of CAM in-vivo with elegant on-line natural abundance isotope discrimination studies, and these also sealed the interpretations of shifting $\delta^{13}C$ values discussed below. Subsequent GCMS and nuclear magnetic resonance (NMR) studies facilitated assessment of the extent of fumarase randomization in CAM itself, and suggested that the

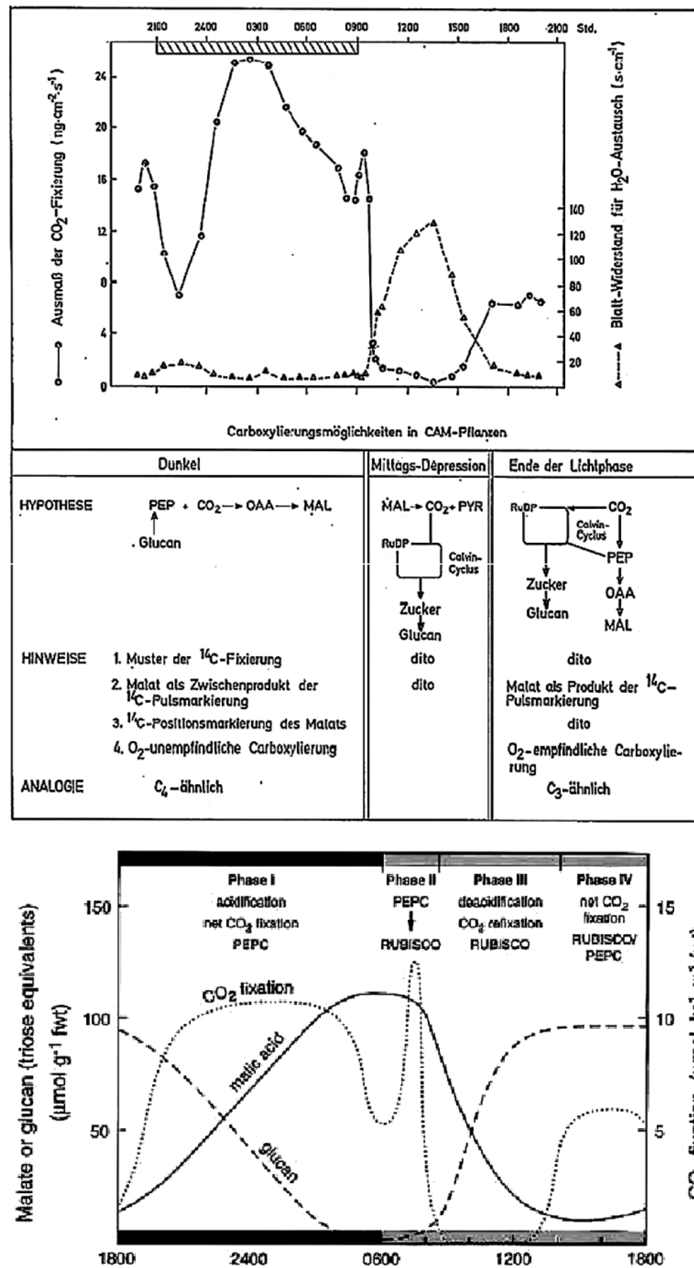

Fig. 1. Origins of the phases of CAM concept. An early summary of evidence (above) for C_4-like and C_3-like carboxylation events separated by deacidification of malic acid (Osmond and Ziegler 1975) corresponding to phases I, IV and III respectively, and (below) from a textbook chapter (Leegood et al. 1997). Phase II, the transition in carboxylation events early in the day, emerged from studies of plants in growth chambers exposed to sudden transitions in light. Perhaps one should also observe phase V, another period of transition in carboxylation events at the end of the day, especially in CAM plants exposed to slowly declining light under natural conditions. Diagrams reproduced by permission of the publishers; Wissenschaftliche Verlagsgesellschaft mbH and Pearson Education Ltd, respectively

equilibration of vacuolar, cytoplasmic and mitochondrial pools of malate changed during long-term exposures to $^{13}CO_2$ in the dark (Osmond et al. 1988). Much still remains to be done to convincingly evaluate these relationships. Although specification of the phases of CAM provided a helpful framework for a better understanding of CAM, it is important to recognize now that CAM can be much more plastic, indeed more fantastic than one then could have imagined (Dodd et al. 2003; Lüttge 2004).

1.3 Biochemistry and diffusion as determinants of the $\delta^{13}C$ value in CAM plants; improved understanding of water use efficiency in C_3 plants

Perhaps the most significant application arising from these studies of labelling patterns in CAM emerged from Marion O'Leary's interest in PEPCase in vivo. It is fair to say that interpretations of $\delta^{13}C$ values in C_3 and C_4 plants prior to 1980 were empirical, and lacked a rigorous mechanistic insight. When we were able to move the $\delta^{13}C$ value of *Kalanchoë daigremontiana* Hamet et Perrier de la Bâthie from about −16‰ to −29‰ by simply changing day-night temperature regimes and water stress exposures of plants in the same cross-gradient growth room of the Madison Biotron (Osmond et al. 1976), Marion became curious. An expert in heavy-isotope effects on enzyme kinetics, he immediately saw the merit of detailed evaluation of component processes (CO_2 diffusion, hydration to HCO_3^- and enzyme catalysis by PEPCase and malate dehydrogenase) contributing to the $\delta^{13}C$ value of CAM malate. From Marion's perspective of enzyme kinetic analyses, the carboxylation process in CAM in the dark was reporting in-vivo, as close as it gets a coupled PEPCase assay in-vitro. Many studies had shown that little else was labelled during dark $^{14}CO_2$ fixation, that there was little further metabolism of the product in the dark, and the isotopic composition of all C atoms in the product could be examined. Furthermore, because most CAM plants show substantial stomatal limitation to CO_2 diffusion, even when stomata are wide open in the dark, isotopic signatures due to diffusion were also readily detected.

Arriving in Canberra one Christmas eve, having been rained-out of a camping and walking tour of New Zealand, Marion went to work on the natural abundance ^{13}C of carbons in malate accumulated in *K. daigremontiana* and *B. tubiflorum* Harvey. His analysis of dark CO_2 fixation in CAM showed us how to sum the biophysical and biochemical components of in-vivo isotope fractionation (O'Leary and Osmond 1980). These insights soon led to a new understanding of carbon isotope fractionation in C_3 and C_4 plants with

much more important consequences. It was recognized that integrated average stomatal conductance could be inferred from changes in $\delta^{13}C$ values in C_3 plants and correlated with water use efficiency (Farquhar et al. 1982). As a result, $\delta^{13}C$ values have been used in breeding programmes to select more water use efficient cultivars of wheat (Condon et al. 1990) and other crops, adding much to the value of marginal agriculture in Australia and elsewhere. At the time we were also engaged in ecophysiological studies on "prickly pear" [*Opuntia stricta* (Haw.) Haw.] that, by the 1930s, had denied access to otherwise productive land over large areas central-eastern Australia (see below). In retrospect, it is a delightful irony that, half a century later, insights from CAM should help advance cereal agriculture in the very same regions.

These insights into the importance of diffusion later led us to predict that the $\delta^{13}C$ value of malate should be somewhat less negative towards the centre of thick CAM tissues (Robinson et al. 1993). Indeed, slow diffusion of CO_2 in thick leaves of CAM plants, with low stomatal frequencies and intercellular airspaces often below 5% (Smith and Heur 1981), is manifest in photosynthetic metabolism in other interesting ways. Maxwell et al. (1997) estimated that intercellular CO_2 concentrations at the sites of Rubisco carboxylation were only 108 μbar in *Kalanchoë daigremontiana* with open stomata in air (380 μbar CO_2) during C_3 carbon assimilation in the afternoon. It should be no surprise then, that we had earlier noted clear labelling of intermediates of photorespiration during $^{14}CO_2$ feedings in phase IV (Osmond and Allaway 1974).

We now have other evidence that the high internal resistance to CO_2 diffusion also seems to manifest itself during decarboxylation in phase III. Chlorophyll fluorescence images of the efficiency of PSII are extremely heterogeneous in phase III (Rascher et al. 2001; Siebke and Osmond, unpublished), and are characterized by randomly arising and fading patches (or fronts) of higher efficiency. The heterogeneity persists during endogenous rhythms in continuous light. These suggest spatial and temporal differences in CO_2 concentration arise behind closed stomata as deacidification in some areas proceeds faster than in others. Remembering that CO_2 diffusion in wet cell walls is likely to be 3–5 orders of magnitude slower than in intercellular air spaces, the interpretation seems reasonable. Although Duarte et al. (2005) have recently demonstrated that lateral diffusion of CO_2 occurs in leaves of *K. daigremontiana* over periods of hours, the patterns observed during deacidification change with time constants of minutes. It seems likely that the smooth curves of deacidification are a product of local heterogeneity in the implementation of the regulatory cascade that control PEPCase sensitivity to malate and other elements of this part of the CAM cycle.

1.4 Regulation of CAM PEPCase in the dark and light; its role in the diurnal rhythms of CAM and in C_4 plants

The desensitization of CAM PEPCase to end product inhibition by malate in the dark and its sensitization to the same process in the light is one of the most elegant, thoroughly and creatively documented, reversible regulatory cascades of a core physiological function in plant metabolism (Nimmo 2000). Early studies by Manfred Kluge (Kluge and Osmond 1971a,b) and Irwin Ting established the distinctive K_m, high V_{max} form of PEPCase in CAM (Ting and Osmond, 1973a) in which G-6-P desensitized the enzyme to the inhibitor malic acid (Ting and Osmond, 1973b). Thanks to Manfred and others, feedback inhibition of PEPCase by malic acid was soon implicated in the regulation of dark CO_2 fixation which tended to decline as malic acid accumulated in the vacuole towards the end of the dark period (Kluge et al. 1980). However, it was the experiments of Klaus Winter during his postdoc in Canberra (Winter 1981, 1982), and those of Jones et al. (1981) in Wilkins' laboratory in Glasgow, that stimulated the search for the PEPCase regulatory cascade. We now know that in CAM high affinity CO_2 fixation in the dark continues in the face of high malic acid contents because PEPCase is phosphorylated and desensitized to inhibition by the accumulating malic acid. In the light, PEPCase is de-phosphorylated, becomes more malic acid sensitive, and is largely prevented from competing with Rubisco in a futile carboxylation cycle during deacidification (Nimmo et al. 1986, 1987).

Damped diurnal rhythms of CO_2 evolution in CO_2-free air in continuous dark, of CO_2 exchange in air in continuous light, and their temperature responses, have been distinctive and enduring features of CAM research (Wilkins 1959; Nuernbergk 1961). The early acceptance of an overriding controller was best summed up by Queiroz (1974). He noted then that *"all the available data on CAM rhythms suggest that even if malate feedback inhibition operates under certain conditions in vivo, this effect should be superimposed on a basic oscillator (of unknown nature) which underlies the coherent operation of several enzymes of the pathway"* and that "control by feedback could be more efficient if applied to an already oscillating system". As is evident above, these thoughtful assertions provoked a determined and remarkably successful assault on PEPCase regulation from the likes of Klaus Winter and the biochemists in Glasgow. However, before these studies changed the way we think about the "CAM clock", a moment of levity intruded into this otherwise serious discussion of matters circadian. The first transmission EM pictures of the photosynthetic organelles in CAM plants were published from Eldon Newcomb's laboratory in 1975. These outstanding pictures also seemed to show a "CAM clock" in elegant physical

reality. The stained, ultra-thin sections revealed an "anomalous microcylinder" that presented a perfectly circular multi-point array in transverse section; quite clearly the 24-point cog of the "CAM clock" in *K. daigremontiana* (Kapil et al. 1975; see insert in their Fig. 13). In fact, the plant material had been maintained by vegetative propagation in the Madison greenhouses for decades, and presumably accumulated a virus (possibly a potyvirus; R. Milne personal communication) the structural proteins of which might have produced the "rifled" cylindrical structure responsible for the multi-pointed (18–24) cogwheel in transverse section.

In reality, explanations of the endogenous rhythms of CAM require nothing less than the careful interpolation and interpretation of the whole of CAM physiology and biochemistry. In those CAM plants that display damped diurnal rhythms of CO_2 exchange (only a handful are known in detail), we must embrace not only the coherent regulation of CO_2 uptake by PEPCase, deacidification and CO_2 release by malic enzyme, but also the refixation of CO_2 by PEPCase and Rubisco (Griffiths et al. 2002; Wyka and Lüttge 2003), as well as compartmentation dominated by malic acid fluxes into and out of the vacuole (Hafke et al. 2003), its relationships to metabolite fluxes among smaller organelles (Kore-eda et al. 2005) and of course, the even more complex coherent regulation of carbohydrate metabolism.

I confess to having long favoured the system view; that if left alone, the intricate network of physiology and biochemistry of CAM will oscillate of its own accord in continuous light and dark, so long as C-resources allow. The rather rapid dampening of the rhythm in CO_2-free air in the dark is almost certainly limited by carbohydrate reserves and respiration, and the numerous oscillations in continuous light in a variety of CAM plants obviously reflect the interactions of 2 carboxylases and differing decarboxylation options. The system view has taken strength from elegant temperature shift analyses augmented by on-line stable isotope discrimination and modelling (Grams et al. 1997), from biochemical and molecular evidence that malate overrides the circadian regulation of the PEPCase kinase (Carter et al. 1991; Borland et al. 1999), and from images of the entrainment of areas of low and high PSII efficiency during oscillations in continuous light (Rascher et al. 2001). Indeed, interpretation of these images in terms of the "*the biological clock as an assembly of coupled individual oscillators*" simply refers to independent nodes of the CAM system isolated by slow diffusion of internally generated CO_2 in a tissue with little intercellular air space connectivity.

The remarkable long-term commitment of Bohnert and Cushman to the molecular genetics of CAM in *M. crystallinum* (Cushman and Bohnert 1999) is now facilitating great progress on the circadian regulation of gene expression behind key components of the regulatory cascade (Hartwell et al. 2002;

Boxall et al. 2005; Hartwell 2005). To paraphrase Orlando Queiroz then, perhaps we really are closing on the nature of the basic oscillator that directs the coherent operation of the regulatory cascades of PEPCase and other enzymes of the pathway. To quote Boxall et al. (2005) now *"these experiments will allow us to finally resolve the nature of the circadian oscillator that controls CAM"*. But it brings little comfort to a committed CAM physiologist to learn that analogues of circadian clock genes from a widely studied, but physiologically undistinguished weed (*Arabidopsis*) can now be recognized in *M. crystallinum* and *K. daigremontiana*. Whatever, it remains a matter of some pride that the PEPCase regulatory cascade discovered in CAM plants subsequently has been shown to apply also in C_4 plants (Jiao and Chollet 1991). It works in reverse in C_4, with de-phosphorylated PEPCase less active and more malate sensitive in the dark, and the primary carboxylase more active in the light following the attention of a light activated protein kinase (McNaughton et al. 1991). Some of the debt for insights into CAM inherited from research into the C_4 pathway in the 1960–1970s, has been repaid.

1.5 Malic acid compartmentation in CAM

Since the study of Kluge (1968), it has been widely accepted that accumulation of malic acid into the vacuole of CAM plants, and its concentration in the cytosol, play central roles in regulation of CAM photosynthesis. I remember wondering why Kenyon et al. (1978) went to the trouble of isolating malate laden vacuoles from *Sedum*; where else could one put 0.5 M free organic acid? Although compartmental analysis using specific activity arguments and isotope exchange methods indicated separate cytoplasmic and vacuolar pools of malate in storage tissue discs (MacLennan et al. 1963; Osmond and Laties 1968; Cram and Laties 1974), direct attack on the compartmentation and tonoplast transport systems for malic acid in CAM was surprisingly slow to emerge (Nishida and Tominaga 1987). Not personally engaged in pursuit of these objectives, my background in organic acids and ion transport made me a keen spectator.

Early physiological experiments (Lüttge et al. 1975) explored turgor and tonoplast fluxes of malic acid as key components of the diel CAM system. At a meeting in Toronto, Canada, Ulrich and I sketched some of the implications of an ATP dependent proton pump for the energy metabolism of CAM on the back of a napkin over a meal in a Chinese restaurant. Surprised by the bioenergetic demands of malic acid compartmentation in CAM in the dark, Ulrich engaged expert opinion and published a speculative paper (Lüttge et al. 1981) that set him on a path to deep engagement

with these processes that continues today. A decade later, and in the days before e-mail, Ulrich and I used a facsimile machine to hastily assemble a progress report on tonoplast fluxes for Harry Beever's retirement symposium. We made light of the notion that Harry may have lost his way in choosing not to pursue his early interest in CAM (Thomas and Beevers 1949), and had in fact overlooked the remarkable transporter properties of the largest organelle of all. Being realists, Harry's friends presented him with an original print from Beseler's *Hortus Eichstettensis*, a print of *Ricinus communis* L.

Holtum et al. (2005b) have provided an excellent summary of the present understanding of proton and malic acid fluxes into and out of the vacuole of CAM plants. Quantitative freeze-fracture analyses of membrane particles and of protein subunit patterns produced convincing evidence for the induction of vacuolar H^+-ATPase and H^+-PP_iase correlated with the induction of CAM (Lüttge and Ratajczak 1997). The picture emerges of a CAM tonoplast with a high density of H^+-ATPases (plus H^+-PP_iase) and low density of anion-selective ion channels (Hafke et al. 2003) with an apparent $K_{1/2}$ of 2.5 mM for malate^{2-} that facilitates passive movement from the cytoplasm down the electrochemical potential gradient at rates adequate to account for the high influx of malic acid into the vacuole. Efflux during phase III is less well understood, but Holtum et al. (2005) cautiously accept that the vacuolar malate carrier in *Arabidopsis*, a homolog of the human Na/dicarboxylate cotransporter (Emmerlich et al. 2003), might account for H^+ and malate efflux from the vacuole.

1.6 Light use efficiency and photoinhibition in CAM plants; the role of CO_2 supply in the avoidance of photoinactivation

If a preoccupation with CAM came to define my interest in the dark reactions of photosynthesis, early collaborations with Olle Björkman, and in the Anderson-Boardman lab in CSIRO, led me to seek links with the light reactions. As described elsewhere (Osmond and Förster 2006), Olle and I had postulated that when stomata close in response to water stress, photorespiratory CO_2 cycling in C_3 plants might continue to generate a sink for electron transport, and thus mitigate photoinhibition. It was a short step to ask whether the high internal CO_2 concentration during deacidification in phase III of CAM contributed to mitigation of photoinhibition, at least during the first part of the day. The application to CAM may have been consolidated during the long night experiments on gas exchange at Carnegie in 1974. These two streams of research came together a decade later when William Adams III

became a graduate student at ANU with the opportunity to undertake a good part of his research in the Desert Research Institute, Reno, Nevada.

The best criterion then for photoinhibition in vivo was the initial slope of the light response curve of photosynthesis at CO_2 saturation. Quantum yield measurements at CO_2 saturation during phase III of CAM proved to be a straightforward matter using David Walker's O_2 electrode system. It was our good fortune that Professor K. Nishida spent some months in RSBS. A Japanese plant physiologist highly respected for his demonstration of nocturnal opening and diurnal closure of stomata in CAM using a viscous flow porometer (Nishida 1963), three generations of researchers collaborated to estimate quantum yields of O_2 evolution in phase III in CAM. By chance, gardeners had planted the highly reflective CAM plant *Cotyledon orbiculata* L. adjacent to the entrance of RSBS in Canberra. With this plant we not only had an opportunity to evaluate the role of internal CO_2 supply, but the ability to decrease reflectance (and hence increase absorption) by simply brushing the wax surface. William's first paper on quantum yield in CAM (Adams et al. 1986) appeared practically simultaneously with the wider survey of O_2 evolution in C_3 plants by Demmig and Björkman (1986). What happened next is now history as the Adams-Demmig-Adams partnership emerged as one of the most impressive teams in modern plant ecophysiology.

William also used *K. daigremontiana* to clearly show that if malic acid pools in CAM were reduced by exposure to N_2 atmospheres in the dark, the extent of photoinhibition was exaggerated (Adams and Osmond 1988). The role of CAM in conserving respiratory CO_2 and mitigating photoinhibition in tropical ferns was soon confirmed by Griffiths et al. (1989). Meanwhile, the extreme light environment of the Mohave Desert and the extensive work of Irwin Ting on the beautiful, but optically opaque beaver-tail cactus *Opuntia basilaris* Engelm, and Bigelow made this plant a natural choice for investigation of photoinhibition on sun and shade exposed sides of the cladodes. I was not much help to the project, once requiring repatriation from the field after falling victim to severe sunstroke while working in Grapevine Canyon, Death Valley. William's 77K chlorophyll fluorescence and quantum yield data (Adams et al. 1987) were interpreted in terms of our then understanding of photoinhibition. Within 2–3 years our understanding of photoinhibition changed rapidly as the importance of photoprotection associated with the xanthophyll cycle became evident. This is perhaps most clearly shown in the later study (Adams et al. 1989) of *Nopalea cochinillifera* (L.) Salm-Dyck (synonym for *Opuntia cochenillifera* (L.) P. Mill.) in Venezuela.

We came full circle in this aspect of CAM when Sharon Robinson showed that removal of the reflective wax from *C. orbiculata* increased the depth

within the tissue to which conversion of violaxanthin to zeaxanthin could be detected (Robinson and Osmond 1994). Her study neatly integrated the concepts of external photoprotection by reflectance, and internal photoprotection by the xanthophyll cycle. Nevertheless, there is no doubt that the CO_2 concentrating mechanism of CAM provides an excellent natural model for the importance of access to CO_2 in sustaining high photosynthetic efficiency in strong light. It is clearly a less ambiguous general model for the role of internal CO_2 generation in mitigation of photoinactivation than our earlier hypothesis for the role of photorespiratory carbon cycling in C_3 plants when stomata close in response to water stress.

1.7 Recycling of respiratory CO_2 in CAM and diel variation in the engagement of cyanide insensitive respiration

Recycling of respiratory CO_2 in phase I is a feature of CAM cycling and CAM idling (Kluge and Ting 1978), but engagement of the CAM pool of malate in tricarboxylic acid (TCA) cycle metabolism and mitochondrial electron transport remains to be assessed in detail. Mitochondria isolated from CAM plants have a high capacity of the alternative oxidase in vitro (Rustin and Quiroz-Claret 1985), but as with all such studies, evaluation of activity and function in vivo remained elusive. The creative insights of Joe Berry, Jim Siedow and Dan Yakir, the technical excellence of Larry Giles and the post-doctoral confidence of Sharon Robinson and Miguel Ribas-Carbo, were directed to meddling with an O_2 electrode attached to a mass-spectrometer made available by a grant from the broadminded North Carolina Biotechnology Center. The team discovered that alternative oxidase activity peaked in phase III of CAM, just as predicted to assure the least adenylate control and maximum TCA cycle flexibility during deacidification (Robinson et al. 1992). Their method proved generally applicable, and has now given better insights into the functional significance of cyanide-insensitive respiration in other plants (Robinson et al. 1995).

When fitted with a gas phase CO_2 electrode, David Walker's O_2 electrode system revealed that stoichiometric nightmares of net O_2 and CO_2 exchanges prevail in all phases of CAM (Osmond et al. 1996). Again, Maxwell et al. (1998) showed that these can only begin to be resolved when the CAM tissues are attached to a mass spectrometer tuned for tracer experiments with $^{13}CO_2$ and $^{18}O_2$. In the course of these experiments, we were confronted with a simple but poorly understood feature of CAM; the extraordinarily high concentrations of O_2 that accumulate in the closed system during phase III conversion of a soluble CO_2 source (malic acid) to soluble and insoluble

products (sugars and starch) accompanied by photosynthetic O_2 evolution. The process could be observed by allowing the CO_2 concentration to oscillate over a narrow range in short light-dark cycles while O_2 concentration increased in each light period (Osmond et al. 1996, 1999). The over-pressure of O_2 was clearly audible when the chamber was opened at the conclusion of each experiment.

We estimated that deacidification of 100 μmol malic acid g^{-1} fwt could generate up to 9 ml O_2 in a tissue with a gas space of about 50 μl ml^{-1}. The high internal CO_2 concentrations generated during deacidification seem adequate to mitigate the oxygenase activity of Rubisco and minimize photorespiration during CO_2 fixation (Osmond et al. 1999; Lüttge 2002), but the O_2 partial pressures that build up behind closed stomata in phase III of CAM are probably higher than in any other living system. One had encountered anecdotes about this phenomenon from time to time, but now we can be grateful to Krätz (2001) Ulrich Lüttge and Otto Lange for pointing us to the discovery of O_2 over-pressure in *Clusia rosea* Jacq. by Alexander von Humboldt, in the field, in 1800! When a cut leaf was illuminated with its petiole in water, the gas bubbles released from the petiole were found to contain 30–35% O_2. The implications for oxidative stress are obvious, but still need to be probed in depth (Broetto et al. 2002). Many investigations of the potential for photo-oxidative stress associated with photosynthetic O_2 evolution in the chloroplast have been frustrated by problems of access, detection of reactive oxygen species, and the remarkable multiplicity of metabolic pathways. Frankly, why would one look beyond the CAM system to discover what really matters in oxidative stress?

1.8 Field CAMpaigns

From the above, it is clear that my CAM research has been largely lab-bound. I was much aware of the advances arising from Irwin Ting's fieldwork, and while in München, made brief excursions to dry sites in the Alps with Professor Ziegler. We were also impressed by the comprehensive ecophysiological and biophysical evaluation of massive CAM succulents being undertaken by Park Nobel. His book (Nobel 1988), complete with cover illustrations provided by the US Postal Service, made it clear that field CAMpaigns should be directed elsewhere. However, there was a time when visitors to the RSBS laboratory were pressed into brief field trips over long distances to study "prickly pear" (Osmond et al. 1979a). Unfortunately, our primitive hands-on equipment made it difficult to avoid the annoying, tiny barbed glaucids of this otherwise benign succulent; one hopes now all is forgiven!

Introduced as a hedge for vineyards in 1846, today's highly dispersed remnant populations of *Opuntia stricta* give little clue to the impact of this invasive CAM plant in central-eastern Australia. By 1930 this succulent had become a noxious weed of immense proportions, having expanded to deny agricultural and pastoral access to an area as large as that of the British Isles, often attaining 500 or more tonnes fresh weight per hectare (Osmond and Monro 1981). As early as 1915 investigators knew they were dealing with something different. A chemical control agent (arsenic pentoxide in 15% sulphuric acid) proved more effective when sprayed at night (presumably because stomates were more open at night), and thousands of farmers knew that cladodes of prickly pear seemed to survive "on air", without roots. However, nocturnal malic acid synthesis and the water use efficiency of CAM in *Opuntia* did not attract much interest among Australian plant physiologists of my generation, possibly because the "prickly pear" problem had been resolved by one of the most spectacular biological control regimes ever established.

Research by Gert Stange, a visual neurophysiologist now working on bio-inspired autopilots for flying robots, revealed that the continued highly effective biological control of this noxious invasive CAM plant depends to a significant extent on exquisitely sensitive CO_2 detectors in the mouth parts of female moths *Cactoblastis cactorum* Berg (Stange et al. 1995; Stange 1997). Ever since their introduction in the late 1920s, female *C. cactorum* have been making boundary layer CO_2 profiles 1–5 mm above any surface upon which they alight during their early evening perambulations. Possibly the most persistent and numerous CAM researchers on the planet, after detecting a surface with net CO_2 influx in the dark, the female *C. cactorum* proceed to deposit eggs on the glaucids in the aureoles of *Opuntia* cladodes, the most uncomfortable but most secure spot on the cladode surface.

There is probably a lot more involved in host plant detection (Pophof et al. 2005) but measurement of nocturnal CO_2 influx evidently remains a good targeting system throughout the range of *O. stricta* in south-eastern Australia (there is only one native CAM plant in the region; *Sarcostemma australe* R.Br.). Larvae of *C. cactorum* simply burrow into the cladodes and totally devour them from within. Millions of tonnes of *O. stricta* were consumed by trillions of larvae and after three damped cycles of devastation and recovery over a decade, *O. stricta* ceased to be a problem (Osmond and Monro 1981). Half a century later, when ecophysiologists began to study the dispersed remnant populations of the CAM invader, we quickly gained an appreciation of the tight population dynamics underlying this unobtrusive, highly effective biological control. Unless clumps of *O. stricta* were isolated by insect netting, *C. cactorum* usually found and devoured its host

before we could return to complete seasonal measurements of CAM (Osmond et al. 1979a,b).

I may have been cured of CAM fieldwork by the *Stylites* expedition of 1982. In an unguarded moment during a discourse on the stomate free cuticles of fossil plants at the Sydney Botanical Congress in 1981, John Raven leaned over and muttered "they must have been CAM then". He was overheard by Jon Keeley, who retorted "I know just where to find such CAM plants". The cover article in *Nature* that followed should have been headlined with the best insight of the trip ("How does Stylites CAMpeat?"; courtesy of Sterling Keeley). However, the remarkable capacity of the sporophylls of this taxon to recycle CO_2 from its peaty root zone via CAM in chloroplast containing cells surrounding the air canals connected to gas columns in living roots was a good story in itself. The fieldwork cure took the form of excruciating headaches (from inattention to altitude in the Peruvian Andes), and a really debilitating stomach problem (from inattention to ethnic culinary style in restaurants) that almost led to a JAL cabin crew being quarantined at Tokyo airport over Christmas.

As it was, Klaus Winter organized my first and only field encounter with CAM in the tropics (Winter et al. 1986), and I regret having been distracted by other things during the "grand era" of tropical CAM ecophysiology. One cannot but admire the results and impact of expeditions to tropical CAMscapes; to the Bromeliads in Trinidad (Griffiths and Smith 1983), to the Clusias in the Caribbean and tropical Americas (Ting et al. 1985) and to the diverse stem succulents of Madagascar (Kluge et al. 1991). These expeditions have stimulated our wider appreciation of CAM plants well beyond the Fensterpflanzen of Carl Spitzweg. The recent history of CAM ecophysiology is remarkable for the ways fieldwork has led to creative laboratory investigations, ranging from use of isolated vacuoles from orchids for transport studies (White and Smith 1992) to the establishment of records such as 1.4 M titratable protons in vacuoles of *Clusia* (Borland et al. 1992) and to records in xanthophyll photoprotection set by *Guzmania* (Maxwell et al. 1994).

As one may sense from Black and Osmond (2003), I thought that my dreams for field work with CAM plants might be realized after accepting the challenge to lead the Biosphere 2 Laboratory to fulfil its potential for experimental ecosystem and climate change research. This was by no means the highest priority for research in the beautifully situated, extraordinary facility in the higher Sonoran Desert, but we set out to examine some system level impacts of CAM under controlled conditions. An enclosed environmentally controlled facility, retrofitted and named in honour of Manfred Kluge and Ulrich Lüttge, Nobel and Bobich (2003) explored the carbon sources for new root growth of *Opuntia* spp. following summer precipitation,

and Rascher et al. (2006) demonstrated that the imprints of the phases of CAM can be discerned in net ecosystem CO_2 exchanges.

The premature termination by Columbia University of its 10-year commitment to the transformation of this unique apparatus, just as its potential for global change research was becoming so clearly evident, will be remembered by many as one great opportunity lost. In a lighter vein, I regret that we did not get to evaluate the havoc that must be wrought on CAM in *Carnegia gigantea* (Engelm.) Britt. & Rose draped each night in suburban gardens of Arizona with garlands of red, white or blue LEDs for weeks at a time during the festive season. Although I pointed out the misinformation on a panel at the Arizona Sonora Desert Museum (that asserts CAM plants have the remarkable ability to split H_2O to O_2 in the dark!) nothing had changed on my last visit.

1.9 Origins of CAM and its future prospects in a high CO_2 world

The evolution of CAM is a question that is best left to the experts. Aside from noticing that the CCM upon which CAM depends simply involves internalizing the CO_2 atmospheres of the Cretaceous, I have not paid much attention to the problem. In an unguarded moment at a photosynthesis congress in Brussels, shortly after the encounter with *Stylites*, and no doubt while still overly impressed with contemporary progress in CAM, I was drawn to speculate that it might represent an ancestral, terrestrial photosynthetic metabolism for all seasons. Now in one's dotage even a firm believer in the principle that one should either put up (experimental evidence) or shut up, may be allowed a little fanciful speculation. If one contemplates the primal cellular requirements of cation–anion balance, pH stasis and turgor in a vacuolated autotroph during metabolism of nitrate (a shadow of *Atriplex* here!), then the anaplerotic, cytoplasmic synthesis and vacuolar accumulation of malate in CAM seems an admirably comprehensive compromise. If recycling of respiratory CO_2 and conservation of water are of further selective advantage to the C-balance of a multi-cellular terrestrial system, we seem presented with the key design elements for CAM. Lüttge et al. (2000) took a step towards evaluation of these possibilities in their demonstration of linkages between the housekeeping function of vacuolar malate transport for cation balance in tobacco under nitrate (but not ammonium) nutrition, and the special role of malic acid fluxes in CAM.

Some more thoughtful commentators now seem not to dismiss the above notions out of hand in their discussions of the physiological and biochemical realities of C-acquisition in aquatic, and subsequently in water limited terrestrial habitats. Keeley and Rundel (2003) entertained the possibility that

the temporally regulated survival attributes of CAM in aquatic and terrestrial habitats may well have predated the more productive, spatially regulated attributes of C_4 metabolism. Sage (2002) made an outstanding case that the developmental, physiological and biochemical analogies between the CCMs in CAM and C_4 metabolism are probably mutually exclusive in functional terms, except perhaps if spatially and temporally separated in *Portulacca* (Guralnick et al. 2002). Unlike C_4 photosynthesis where the CCM is associated with the vasculature and defined by suberin, the CCMs of CAM plants cannot be expected to leave much of an anatomical signature during fossilization. On the other hand, as Uwe Rascher has pointed out, woody CAM plants that rarely if ever engage in CO_2 assimilation in phase IV, might provide long lasting less negative $\delta^{13}C$ signatures that are relatively unresponsive to environment variables.

Whatever, the safe position in these matters is to retreat to the notion that CAM is a remarkably flexible, niche-filling form of photosynthetic metabolism originating in many families that is found now in some 16–20,000 ancient and modern aquatic and terrestrial species. Crayne et al. (2004) make the point that terrestrial and epiphytic niche differentiation seems to have been important during evolution in different genera in the Bromeliaceae. In this "Arabicentric era" of plant biology, Hartwell (2005) speaks for all in this field when he asserted that *"we overlook such valuable adaptations (as CAM) at our own peril in the face of current predictions of global warming"*. In spite of the efforts of the dominant mammal (humankind) to restore Cretaceous-like atmospheres on Earth by profligate combustion of fossil photosynthates, the CCM of CAM may confer advantages in many hot tropical and seasonally arid climates for some time to come. Perhaps one should be concerned for future biological control of "prickly pear" because, as atmospheric [CO_2] continues to increase, the ability of *Cactoblastis* to detect inwardly directed CO_2 gradients over *Opuntia* cladodes at night is compromised (Stange 1997). Perhaps we should be more concerned with the possibility that promotion of profligate use of fossil fuels may be seen by some administrations as a convenient way of burning much of the evidence for biological evolution itself!

1.10 A view from over the hill

I concluded an earlier review of CAM with a quote from Nehemiah Grew that promised research would continue to be an uphill quest (Osmond 1978). Fortunately perhaps, I seem to find myself wandering the foothills still, confronted with so many questions in CAM demanding to be addressed that one scarcely knows where to turn. A new set of generic questions seems

to turn up with each forward step, and we remain somewhat blind to several major issues. For example, we have been preoccupied with elucidation of the signature metabolism of CAM, with malic acid synthesis and degradation, and have paid less attention to what may be even more sophisticated regulation of carbohydrate metabolism and its relation to growth. The fundamental question of how CAM plants preserve carbohydrate reserves in the light for acid synthesis in the dark, while at the same time providing carbon for growth remains enigmatic. In particular, the natural abundance stable isotope evidence for discreet pools of carbohydrates engaged in dark CO_2 fixation and growth (Deleens et al. 1979) challenges all present models of metabolic compartmentation. It is astonishing, but the first detailed diel carbon allocation budgets of CAM plants seem to have been published by Borland (1996) and Borland and Dodd (2002).

Two of my earliest PhD students made important contributions to carbohydrate stoichiometry in CAM (Sutton 1975a,b) and to gluconeogenesis (Holtum and Osmond 1981); studies that have continued with subsequent students (Christopher and Holtum 1996, 1998). Carbohydrate metabolism in CAM became a cornerstone in Clanton Black's lab, with discovery of the novel pyrophosphate dependent 6-phosphofructokinase (Carnal and Black 1979) and recognition that sugars in the vacuole, rather than starch in the chloroplast, are the source of substrates for acidification in pineapple and other species (Black et al. 1996). Clearly, major issues of carbohydrate compartmentation and transport in CAM, such as vacuolar sucrose fluxes (McRae et al. 2002), glucose and glucose-6-phosphate fluxes into and out of chloroplasts, and the unusual regulatory relationships with fructose 2,6 bisphosphate (Fahrendorf et al. 1997), need close attention. Given the revolution in our understanding of starch metabolism in leaves (Smith et al. 2005), the unusual demands of CAM may soon be placed in context (Holtum et al. 2005).

When do CAM plants actually grow? Years ago, Bill Allaway weighed *K. daigremontiana* grown in controlled environments in the Canberra Phytotron that were designed to vary the amount of CO_2 fixed in phase IV, and as expected these plants grew faster than those confined to CO_2 uptake in phase I. Ignoring for the moment that we do not know much about the diel growth properties of leaves from more than a handful of tame C_3 plants that mostly grow at night, we have been surprised to find that CAM plants grow in the day during phase III (Gouws et al. 2005). A few moments of reflection were enough to convince us that this made a lot of sense. During phase III carbon skeletons need to be conserved in starch for the next night of malic acid synthesis, but in the absence of phase IV net CO_2 fixation, 25% of malate carbon could become available for growth and maintenance. Moreover, because turgor peaks in phase III, and cytoplasmic

pH declines, this phase of CAM seems the most propitious for growth of leaves and cladodes.

The ecophysiological literature suggested a further test, using the conveniently planar leaves of *Clusia minor* L. that switch from CAM to C_3 and back again in response temperature and vapour pressure manipulations (Schmitt et al. 1988). It was at this point we were reminded that ecophysiologists in general are prone to study fully expanded (non-growing) mature leaves, that not much is known of CO_2 fixation in young expanding leaves, and that we now need to revisit the whole mysterious citric acid metabolism of *Clusia.* A recent comprehensive analysis of nocturnal acid synthesis and carbohydrate consumption in strongly CAM *Clusia hilariana* Schlecht demonstrated a remarkably tight sugar-malate dominated stoichiometry (Berg et al. 2004). However, the sole pulse-chase study of these processes (Olivares et al. 1993) leaves open many questions of citrate metabolism that still need to be explored C atom by C atom using $^{13}CO_2$ and GCMS (Osmond et al. 1996). We also now need to study source-sink relationships in growing photosynthetic tissues in CAM plants, taking cues from *Opuntia ficus-indica* (L.) P. Mill. (Wang et al. 1988) and the carbon budget experiments of Borland and Dodd (2002).

Not much of the above research has appeared in generalist journals now held to be of high impact by the "accountants" who now define the parameters for academic promotions and award of competitive grants. Rather, stalwart plant biology journals, served by broadminded editors and reviewers, have facilitated the reporting of CAM research. Consequently, one is no longer surprised to find the feats of CAM physiology and biochemistry cited in headlines in higher impact journals justifying attention to particular genes in *Arabidopsis* (Emmerlich et al. 2003). Preparation of this chapter has reminded me again of just how much there is in CAM that is new, sometimes entirely unexpected and highly specific (Epimashko et al. 2004), but also much that is relevant and indispensable to mainstream plant biology (Hafke et al. 2003; Boxall et al. 2005). This functionally distinctive but economically undistinguished sector of plant biodiversity has added significantly to our genetic, biochemical, biophysical, physiological and ecological understanding of plant biology in general.

Some 40 years of peripatetic engagement in CAM research, from privileged research positions in RSBS at ANU, in Reno, at Duke and briefly in Columbia, have left me with several enduring perspectives:

- First, in all the glorious functional biodiversity of plant systems, one can rarely predict where the next significant insight may arise. The huge impact of ecophysiological studies of tropical epiphytes and stranglers on

widening our understanding of CAM beyond the Crassulaceae is a case in point. Although it was clear that research in CAM would contribute much to the understanding of PEPCase regulation, tonoplast malate translocators and circadian rhythms, it was less obvious that one could have anticipated its contributions to breeding water-use efficient crops, assessment of alternative oxidase in-situ, or the still to be exploited potential of CAM in studies of oxidative stress.
- Second, the collegial networks so critical to progress in research know no boundaries. Even at times when it seemed that different views of the same phenomenon had become polarized, common ground ultimately emerged. Witness for example, the insights that continue to emerge from application of diverse biophysical and molecular genetic approaches to circadian rhythms in CAM (Borland et al. 1999; Lüttge 2000; Nimmo 2000; Hartwell 2005).
- Third, it becomes clear with the passage of time that one's efforts must be directed to sustaining individual creativity, collaborative activity and achievement among younger colleagues.

Is there a bottom line? Several of my mentors in research offered outstanding proof, in their time, of Medawar's axiom; "*If politics is the art of the possible, research is surely the art of the soluble. Both are immensely practical-minded affairs*". They bridged the apparent divide between research and politics with spectacular solutions that enhanced the possibilities for science. Clearly, research politics have not been my forte. Although I have striven, I have not prevailed, in spite of outstanding opportunities to change the ways we think about things, and go about them. In the end, one remains simply grateful to many companions in CAM, and in other pathways, whose integrity and creativity have made his career in botanical research so much more fun, so much more fascinating, and possibly somewhat more enduring, than all the other efforts.

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