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C₄ Photosynthesis: Thirty or Forty Years On

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The C₄ pathway has served as a rallying point for so many creative contributions, a focus for attention from so many outstanding researchers, that any attempt to set the context 30 or 40 years on is doomed to offend the majority of one's colleagues. However, there is one colleague whose personal research has set the standards to which we all aspire, and rarely attain; whose achievements have been applauded for more than a quarter of a century. Hal Hatch has brought great distinction to Australian plant science.

This preamble deals with what is perhaps Australia's most significant contribution to modern plant sciences research. It is certainly one of the main reasons why plant sciences research over the last decade or so has had more influence internationally (measured by relative citation impact), than any other field of biological research in Australia (Bourke and Butler 1994; Osmond 1995). Although we may take pride in this quantitative evidence, I believe we share a deeper sense of achievement in C₄ pathway photosynthetic carbon metabolism. This deeper sense is reflected in the neatness, the comprehensiveness, that accompanied the unfolding of our understanding in this field. We can identify with Medewar (1968) that, above all else, researchers value discoveries first for their explanatory value, second for their clarifying power 'the degree to which they resolve what has hitherto been perplexing' and third, for the feat of originality involved, 'the surprisingness of the solution to which it led'.

Thirty or 40 years on, it is good to reflect on where we have come from, what has been achieved and, as this Symposium has defined, where we will go next. One acknowledged starting point is an incidental observation and an inspired guess of Haberlandt (1884), whose anatomical insight provoked a more comprehensive understanding of photosynthetic carbon metabolism in all its forms. There is no question that the insights and the confidence gained through progress in C₄ photosynthesis research led to a mature view of photosynthetic carbon metabolism in C₃ and CAM plants as well. Our perception of leaf photosynthesis has been refined through the integrative philosophy, and growth of research capability, that accompanied the

unfolding of the C₄ pathway. We are now presented with a plethora of well-defined new problems that tax our creativity in fields of gene expression, cell biology, ecophysiology and evolutionary biology, all arising from a desire to understand this remarkable pathway.

In a musical contribution to an early C₄ pathway conference, it was heralded that Roger Slack wanted a 'C-through plant' (Hatch *et al.* 1971). After 30 or 40 years, the essence of the C₄ pathway is so transparent that it gives insight into some of the big questions in plant biology with the promise of more to come. Looking back, it seems natural to deal with the growth of research in C₄ photosynthesis as phases of a generalised biological growth cycle.

Induction Phase (Before 1965)

Several markers stand out in the prolonged induction phase of C₄ photosynthetic research. There are likely to have been many others. Although it is widely recognised that Haberlandt (1884) bequeathed the term Kranz to describe the wreath-like arrangement of mesophyll and bundle sheath cells characteristic of many C₄ photosynthetic systems, his intuitive '*oder ob eine noch unbekannte Arbeitsteilung zwischen den Chloroplasten der Kranz- und jenen der Scheidenzellen dabei im Spiele ist, bleibt dahingestellt*' is less well appreciated. One of the neatest elements of C₄ photosynthesis is the now well-known division of labour (*nun wohlbekannte Arbeitsteilung*) between chloroplasts in the assimilatory tissues of the mesophyll and bundle sheath. The spatial separation of the processes that permit the working of a CO₂ concentrating mechanism in C₄ plants, most economically described by Karpilov (1970) as '*cooperative photosynthesis*', was quickly accepted into textbooks in the broad context of a significant biochemical adaptation (Hochachka and Somero 1973).

Perhaps the most specific early markers in the long induction phase of C₄ photosynthesis are those signalled by Burr *et al.* (1957), the frequently overlooked and relatively inaccessible report of Karpilov (1960), and the first, widely accessible, report of Kortschak *et al.* (1965). As Andy Benson testified during this conference, and as the last figure

in Bonner (1950) has enshrined, doubly-labelled C_4 -dicarboxylic acids were perplexing early products of early studies of photosynthetic $^{14}CO_2$ fixation in the 1950s and 60s. Given the Medewarian embrace of the Benson–Calvin cycle at that time, it is not surprising that anomalies uncovered by researchers in Hawaii (colonial USA) and in Kazan (colonial USSR), attracted little attention.

At least one graduate student at that time, probing the metabolic origins of dicarboxylic acids involved in leaf ionic balance, and aided by decarboxylation techniques then used by the Wine Research Institute in Adelaide to measure malic acid in wine, was perplexed to find singly labelled malate and aspartate as the earliest products of photosynthesis in saltbush (Osmond 1965, 1967). Thirty years ago I recall standing in front of the chalk board in George Laties' laboratory at UCLA with James Bassham, there experiencing first-hand the pervasive influence of the Benson–Calvin cycle as I discussed evidence for the primary labelling of C-4 in malic acid. Who was interested in the path of carbon in *Atriplex* anyway?

Evidently, things had been somewhat more lively at the Australian Society of Plant Physiologists meeting in Hobart in 1965 when Hatch, Slack and their students broke the news. To my lasting regret I did not meet Hal at the time, having been repatriated from the conference with an extraordinary bout of 'flu, to recuperate and finish a PhD thesis. Joe Wiskich, who had helped turn the stopcocks on the evacuated flasks we had used to draw $^{14}CO_2$ rapidly through a leaf chamber (designed by Heath-Robinson and Associates, and operated outdoors in Adelaide so as to maximise dilution of ^{14}C with atmospheric ^{12}C in the event of leaks), reported results that neither of us fully understood at the time. Hal was reported to have said 'yeah-yeah, very interesting'.

Early Log Phase (1966–1976)

Edwards and Walker (1983) identified the application of pulse–chase–labelling as a distinctive starting point for the unfolding of the C_4 pathway. Although Burr *et al.* (1957) referred to transfer of C_4 acid label to sugars, this technique and systematic analysis of labelling patterns, which became the hallmarks and principal criteria for the diagnosis of C_4 metabolism, were championed by Hatch and Slack (1966). These simple techniques remain the least equivocal tests for C_4 photosynthesis, tests upon which many claims have failed, and upon which many fascinating anatomical indications continue to be confirmed (Ueno *et al.* 1988).

Over a period of 5 years or so, a spectacular array of observations in plant systematics, as well as leaf anatomy, cell biology, biochemistry, photosynthetic gas exchange and ecophysiology, all fell into place. Although experiments with the radioisotope ^{14}C continued to play a major diagnostic role, advances in enzyme purification and assay, immunocytochemistry, CO_2 and H_2O vapour exchange measurement, and ^{13}C stable isotope discrimination at

natural abundance, all stimulated the exploration of what Black (1971) described as the '*manifold consequences*' of differentiating between C_3 and C_4 plants.

There was a time, when John Downton worked in the Research School of Biological Sciences, and when Olle Björkman spent his first sabbatical in Canberra, that the track up the hill to CSIRO became known as the C_4 pathway. The atmosphere of early log phase was perhaps best summed up by Paul Hattersley (1976) in the introduction to his PhD thesis: '*This rather exhaustive review, which occupied most of my time from November 1973 to late 1974, has now been extensively superseded, and many of the topics I considered have since been dealt with in symposia and reviews. In addition, I have accumulated more than 250 relevant papers, which have been published in the last two years.*'

As peripatetic young investigators, we may have made semantic contributions to the notion of C_3 and C_4 plants (Osmond *et al.* 1969). However, I recall most fondly the 'crossover' perspectives from the ion transport studies of the root cortex by Pitman (1965), that motivated consideration of the metabolite concentration gradients driving cooperative photosynthesis (Osmond 1971), and from light dependent ion transport in *Nitella* (MacRobbie 1965) that helped demonstrate the ability of PSI light to sustain the chase of 4-C label in PSII deficient chloroplasts of *Sorghum*, but not in PSII replete chloroplasts of *Atriplex* (Osmond 1974). Recognising that Canberra is a small town, and that CAM plants offered opportunities in comparative photosynthetic metabolism, Hal Hatch and I collaborated briefly to review compartmentation and transport in C_4 photosynthesis (Hatch and Osmond 1976) before I plodded other paths.

Late Log Phase (1976–96) and Beyond

It would be a brave reviewer indeed who claimed to date the maturing of C_4 pathway research with any precision, because the waves of its influence in photosynthetic research extended rapidly and widely. For example it soon became the core of a comprehensive treatment of ecophysiology, illustrated by much more than one needed to know about *Atriplex* (Osmond *et al.* 1980). It soon spilled over into comprehensive textbooks of photosynthetic carbon metabolism (Edwards and Walker 1983). When Lovelock (1988) factored C_4 plants into the past and future of Gaia, this sector of plant biology had clearly arrived.

To this bystander, late log phase research in C_4 photosynthesis has been marked by an explosion of studies into exquisite metabolic regulation, perplexing developmental problems, C_3 – C_4 intermediates and the arrival of successful transformation systems, all of which are dealt with in this Symposium. Even for one more focused on the environmental, rather than the molecular, implications of

C₄ metabolism, recent issues of journals testify to the current good health of C₄ pathway research. Perhaps reflecting the anatomical origins of the field, and reminiscent of the speculations of Wood (1923), Lal and Edwards (1996) show that centripetal chloroplasts of maize bundle sheaths adopt a centrifugal configuration under water stress, thereby suggesting interesting problems of metabolite pool size and diffusion gradients. Ueno (1996a,b) has comprehensively analysed the ultrastructural differentiation and enzyme compartmentation that accompanies C₃-C₄ induction following submergence and emergence, respectively, in *Eleocharis vivipara*. Biochemically and genetically intractable, this plant nevertheless promises extraordinary insight into environmental control of development in the photosynthetic apparatus.

Having resolved the problem of photosynthesis in the present low CO₂ atmosphere, effectively by recreating an internal high CO₂ atmosphere for Rubisco, equivalent to that prevailing during angiosperm evolution in the Cretaceous, C₄ plants seem destined to be with us for some time to come. In spite of accelerating climatic change (Henderson *et al.* 1994), C₄ plants are likely to remain well represented amongst our worst weeds, our most productive crops, and our most stress tolerant plants. This will ensure that these plants will remain in the forefront of even the most overtly utilitarian research management plans.

About 6–7 million years ago *Sivapithecus*, a relative of today's tree-living orangutans disappeared from the fossil record, probably because its C₃ trees were replaced by C₄ grasses on the Potowar Plateau of Pakistan (Quade *et al.* 1989). The ecological success of C₄ grasses seems to have brought some of our ancestors down to earth, just as the success of C₄ research seems to provoke intervention from our keepers. There seem to be some who feel that C₄ researchers, like *Sivapithecus*, should climb down from their achievements, in the mistaken belief that we are approaching stationary phase, and face extinction. Whatever the real agenda, I hold no fear for the future of research with C₄ plants. Those who feel that the old culture of C₄ research is approaching stationary phase have to concede that it already pervades new research cultures, now labelled as gene expression, cell developmental biology, and so forth. Even if this Symposium reveals that some of these subcultures are experiencing prolonged induction phases, the curiosity intrinsic to research with C₄ plants is likely to ensure that, in unpredictable ways, these too will soon grow into new cultures of research.

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