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Personal perspective/Historical corner

'Tell me where all past years are' *

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Abstract

This is about a young man who wished to go to sea like his father and finished up, instead, in photosynthesis. It describes how he served his apprenticeship in England and the United States and how he was then lucky enough to find himself in the laboratory of Robin Hill, one of the all-time greats in this field. It discusses some of the events that led, via mitochondria in castor beans and carboxylating enzymes in Crassulacean plants, to the isolation of fully functional chloroplasts and the manner in which the first polarographic measurements of CO_2-dependent O_2 evolution contributed to present understanding of the movement of molecules through the chloroplast envelope. It describes some of the problems with materials and apparatus which were commonplace forty years ago and reflects on the advantages of working in foreign places and the pleasures of becoming a member of a truly international community.

'Like a snail, unwillingly to school'

An illustrious French entomologist has a lot to answer for. That and the war. I was already eleven in September 1939 and, at least in the north of England (where I was about to start attending the South Shields High school for Boys), there was a long hiatus while air shelters were built and the younger teachers went off to fight. Mercifully, although television had started in Britain in the 1930s, that too was put on one side while more important matters held sway. So what was a small boy to do apart from playing football, getting into mischief and wondering what little girls were all about? Reading became paramount. By the time that I was four, long before infants' school, my mother (Dorothy) and my 'mad' aunt had taught me to read, thereby giving me the finest gift that any child could receive. I learned to read fast and to read widely. That is how I 'met' Jean Henri Fabre, happily in translation, because tuition in French was now in the hands of a lady of uncertain age, who preferred chatting about the problems of wartime shopping to teaching. Fabre (1823–1915) was an outstanding scientist of his period (Teale 1949). He also had a rare lucidity of communication, guaranteed to enthral any adult reader, let alone a child looking about him with fresh eyes. He immediately raised my standing amongst my schoolboy peers by teaching me how to make touch-paper. At the time we enjoyed making smoke bombs out of old cinematograph film which had somehow come into our possession. Once ignited, tightly rolled pieces of film produced a very satisfactory amount of smoke and, when generated in the wrong places, an equally satisfactory response by our elders and betters. Ignition was a problem however and touch-paper, allowing time for retreat, the answer. Fabre had described how the 'efflorescence' that emerges from cellar walls is composed (I still don't know why) of potassium nitrate. Cellars were largely unknown in South Shields but, by then, there was no shortage of air-raid shelters. The feathery white growth on their walls was duly collected and dissolved in saucers of water through which we ran strips of newspaper. As soon as these strips were dried we were in business. Friends, once sceptical, stayed to applaud. Adults lamented and threatened dire consequences. I acquired an undeserved reputa-

* Written at the invitation of Govindjee

tion as a fledgling chemist. Understandably, despite an excellent chemistry teacher, I didn't want to become a chemist. I wanted to go to sea like my father (Cyril), who in his youth had travelled to the far corners of the earth in a succession of tramp steamers. In fact if my father had still been a ship's officer at that time he would have been lucky to survive the war. Knowing this full well, he would not hear of his only child embarking on a sea going apprenticeship at a time when it was still possible for merchant ships to carry boys of fifteen to their deaths. This left me in a quandary. Staying at school meant taking the 'Higher Certificate', an examination that demanded competence in three principal subjects. I sought to take chemistry, biology and physics but physics came with a requirement for mathematics and some quirk of time-tabling and under-staffing meant that chemistry, my first love, had to go. A major consolation was the inclusion of 'English Literature'. During this period it was very unusual for English children, already embarked upon science, to be allowed to study literature. For me, it added to a love of Shakespeare and to my native language which I have never lost. This and a changing world of language has been central to my life. It is a matter of immense regret to me that I never acquired competence in a language other than my own (although I can get by in North American). At the same time, I have found it an immense and humbling privilege that English has become so universally the language of science that I have been able to communicate as freely with friends and colleagues across the world as I have at home. Indeed, as a traditional purist I am increasingly dismayed to find that the quality of written and spoken English is higher in the overseas scientific community than it is amongst many native speakers. As a Welsh friend once rightly said, his English was better than mine because he had been taught it at school whereas I had only 'picked it up'.

'Full of strange oaths'

Leaving school in 1946 with modest training in science there was the question of what to do next. One school friend happened to be the son of a pharmacist and came therefore from a family which had experience of universities. Without such a background none of the rest of my immediate circle had even contemplated the possibility of going on to university. Neither, I am bound to conclude, can our teachers have believed that we had enough ability to benefit from such an unlikely step. However, swept along by the pharmacist's son, four of us applied to read botany at King's College Newcastle (then part of the University of Durham). What impelled the others towards botany I can't tell. Chemistry would have been my natural choice but then, as I have explained, this was precluded by accidents of school time-tabling. In the event, it scarcely mattered because I was called upon to serve in the armed forces 'for the duration of hostilities only'. Of course, in 1946, 'hostilities' had already ceased but His Majesty's Government pursued its own inane agenda, just as Her Majesty's Government does today. Naturally I was too young to appreciate the fact that, had hostilities not ceased I might not be writing this now. So many, just a year or two younger than me, had endured all manner of dangers and privations which my generation was to be spared. So far as I was concerned, however, being handed over to the tender mercies of The Royal Navy meant incarceration for two years of my young life in conditions more arduous, so far as I can gather, than those currently imposed on prisoners in British jails. So there was rigorous discipline, corporal punishment for trivial offences, little food (and none that was fit to eat) and most of all cold. For three months during the winter of 1947, still the coldest in Britain this century, I was to spend 12 hours a day, in four hour 'watches' on guard duty, often rooted to one spot outside the main gate. Much of this was common experience. Many of my generation, who survived the war in continental Europe as children and teenagers, had experiences beside which mine pale into insignificance. I dwell on it now simply to explain why, if it were not already in my genes, I have (as well as an immense contempt for most politicians) acquired an ingrained skepticism which is every bit as useful to a practising scientist as it was in the days of Robert Boyle (1661).

'Naval' service (I was in the 'Fleet Air Arm' but neither sailed nor flew) was not without its compensations. Naturally, there were lasting friendships of the sort that are formed amongst fellow sufferers in airports and the like. Strangely enough, there was also a continuation of my scientific apprenticeship. During the war, the Navy had discovered that young men with a grounding in physics became very confused when they were confronted with radar. To them, mechanical short circuits which constituted electrical open circuits seemed to defy logic. Conversely, those with a biological bent took these matters in their stride, perhaps because they knew no better or perhaps because they already believed that electronic devices, like living

organisms, are intrinsically perverse and beyond full understanding. So I trained for a year and a day as a radar mechanic. Of course, once trained, I was never asked to use my training for naval purposes but at least I had an early introduction to the theory of electron transport and electrical devices which was eventually to hold me in good stead.

King's College, Newcastle

In October 1948 I was granted an early release from the Navy to allow me to start my studies at King's College rather than having to wait until the following year. I counted myself doubly blessed because many of my contemporary conscripts were told at precisely the same moment (during the Berlin airlift) that they would have to serve an extra three months 'in order to allow us to give an extra thump on the conference table' (a threat, to our minds, which might have convulsed the Red Army with laughter). Release and university were utter bliss. There were no lectures before 10 in the morning and rarely more than one a day. As in an older tradition, students were there to *read* for a degree. Ninety percent were ex-service, some with many years of real war service. None, or so it seemed, who would ever again wish to be told what to do by anyone in authority. 'Sports jackets' and 'flannels' were *de rigeur* but convention bowed to such extravagances as pink shirts and purple ties. With an ex-service grant, I was rich enough to drink beer with my friends every Saturday night provided that I did without coffee breaks for the rest of the week. Advised to take a four year course (rather than the normal three) in order 'to catch up', I repaired my unavoidable school deficiency with three years chemistry. Ever conscious that I had been given a second chance, I worked far too hard. This became a life-time pattern and I often wish that I could have had the courage of those who rightly recognized that time should be reserved, as my friend Dhani (PN Avadhani) once put it, for 'sitting under a tree and playing a flute'.

Approaching my final examinations I was told, but found it hard to credit, that some of us might not only be invited to stay on and do research but would actually be paid for that privilege. Sure enough, in 1952, I found myself in the august presence (Figure 1) of Professor Meirion Thomas (to whom, with TA Bennet-Clark we owe the term 'Crassulacean Acid Metabolism'). By this time he, and colleagues like Stan Ranson (Figure 1) and Harry Beevers, had established, without the help of ^{14}C, that the malic acid which accumulated in *Kalanchoe* leaves in the dark did so as a result of dark fixation of CO_2 (Thomas 1947). Calmly inviting me to identify the carboxylating enzyme responsible for this process, as a suitable task on which to base a PhD thesis, Thomas tempered this proposal with some mention of a less demanding alternative which he would keep in reserve should failure necessitate rescue. He was right to be cautious on many counts, not the least of which was my total lack of experience. The department had no cold-room, no refrigerated centrifuge, no spectrophotometer and a pH meter which paid more attention to the static generated by nylon clad women students than to hydrogen ion concentration (Walker 1992a). No one in his group had ever worked with enzymes. Of course all of this was in the old tradition of throwing students in at the deep-end and assuming that some would survive. In the event I suppose that I must have come perilously close to drowning. Certainly when I was recalled to the august presence some eleven months later I was near to despair and convinced that I was totally inept. But rescue was at hand.

'Hail, hail to old Purdue'

Harry Beevers, late of Newcastle and Oxford, now recently established in Purdue University, Indiana was, it seemed, looking for a research assistant. Even in those days, $120 a month was hardly a king's ransom and, as it later turned out, almost exactly the same wage that was paid to the man who spent an hour each day watering the plants in the 'Pierce Conservatory' adjoining Harry's laboratory. Not that salary even crossed my mind. I was being offered an opportunity to work with an outstanding scientist, barely four years older than myself. Not only that, I was to go to the 'land of opportunity', already made familiar (or so I thought) by so many Hollywood epics. Getting to the United States at that time (1953) still demanded some initial formalities which now seem scarcely credible. At the United States Consulate in Newcastle I had to have my fingerprints taken, declare that I was not a member of the Communist Party and that I had not been convicted of any criminal offence. In addition, three documents were required. One a certificate to say that I was free of venereal disease, a second the results of a full medical examination, and a third (which was eventually to be studied by immigration officials in New York) a full chest X-ray on film (the like of which I was not to see again until I started to

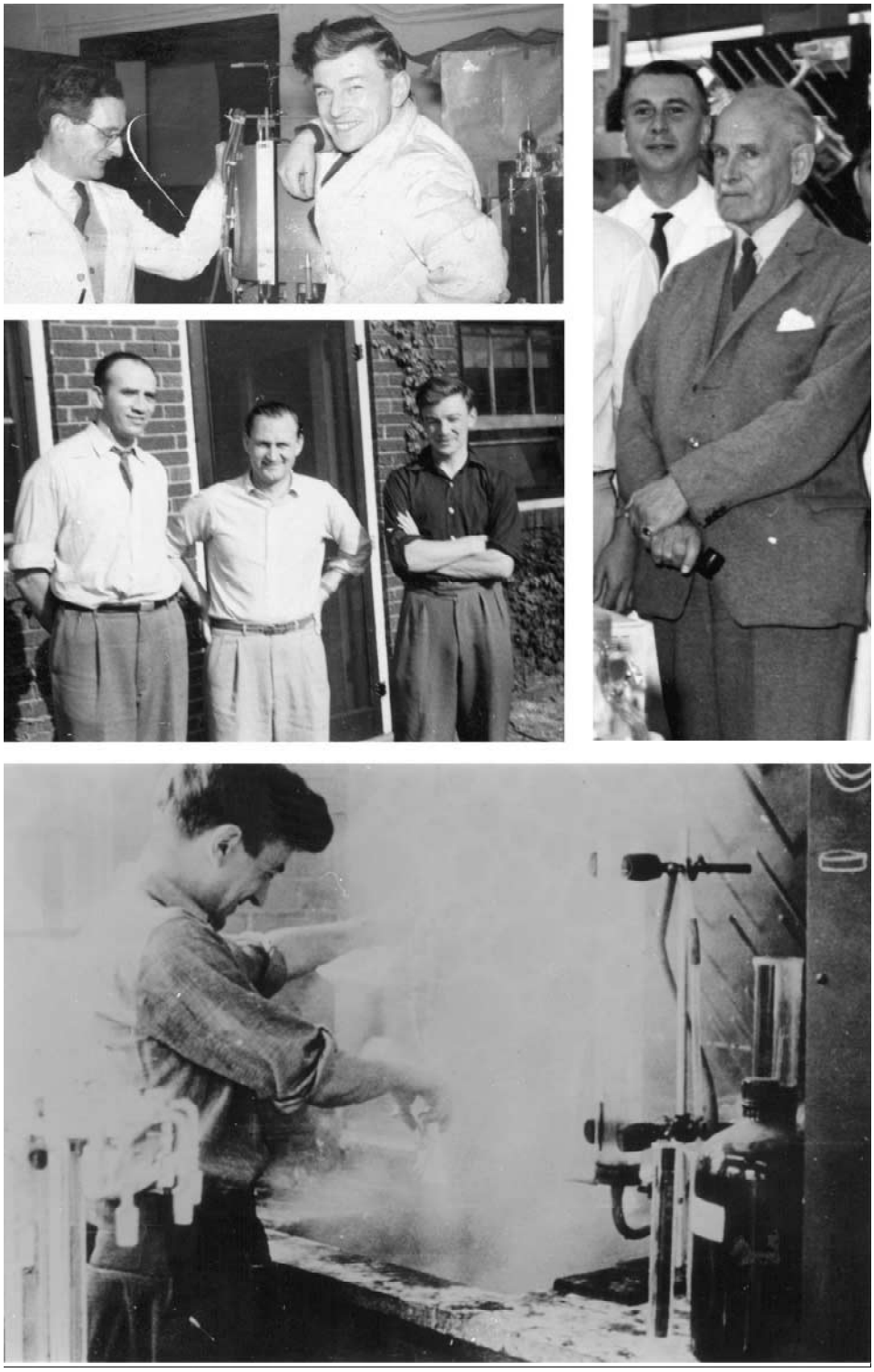


Figure 1. King's College, Newcastle, and Purdue University, Indiana, in the 1950s. *Top left:* Stan Ranson and myself in the 'Harrison Laboratory' at Newcastle. *Top right:* Meirion Thomas. *Middle left:* George Fritz, Harry Beevers and myself at the door of the 'Pierce Conservatory'. *Foot:* Myself cleaning 'Warburg flasks' by holding them under a jet of steam and boiling water in Harry Beevers lab.

use C^{14} some five years later). What bothered me most was that these documents cost me one, two and three guineas, respectively (a total equivalent to about US $10 or 84 bottles of Newcastle Brown Ale at 1953 prices). This happened to be precisely the sum that I had just received for six weeks teaching assistance in university practical classes. Commenting on this to Professor Thomas I was tartly reminded that, at my junior and inexperienced level, such remuneration was undoubtedly much more than I was worth.

My journey to Purdue, courtesy of the Fulbright Foundation (to whom so many owe so much) literally took me into another world. I travelled by sea; cheaper, then, than by air (a passage on 'The Queen Elizabeth' a mere £60). Then via Grand Central Station and a whole compartment (remarkable, to me, for the facilities that it offered) on an overnight train to Indianapolis and on to Lafayette. Harry Beevers (Figure 1), whom I had yet to meet, was not at Purdue when I arrived but on his way back from summer work at the Brookhaven National Laboratory on Long Island, via Madison Wisconsin, where I was asked to join him at my first meeting of the American Society for Plant Physiology. There too, I was to meet Marty Gibbs and start the sort of enduring friendship that would take us to each others homes in the years to come.

Harry was the best thing that could happen to an apprentice scientist. His then minuscule laboratory was a masterpiece of well planned efficiency. His sense of humour enlightened every passing contact. His scientific record speaks for itself. There was also an atmosphere that radiated confidence. Handed a bag of castor beans and unlimited access to a refrigerated Warburg manometer, no possibility of failure was ever even remotely contemplated. On the other hand West Lafayette, Indiana, and Old Purdue (as in 'Hail, Hail to old Purdue') was a bit of a shock. Inevitably, it was not quite as I had imagined. Like many naive expatriates before me, I had supposed that Americans would be more or less like the English. After all I had hitch-hiked through France and Italy when I was still at school and concluded, from that limited experience, that people must be more or less the same the world over. Surely the Americans, speaking my own language and sharing at least some degree of common heritage, would seem even more familiar. On the contrary, marvellous and hospitable as I found the 'Hoosiers', they were as alien to me as I must have seemed to them. I soon discovered that, like my fellow Europeans, I had been brought up to hold my knife and fork in the wrong way, that people actually drank *cold* tea and that if I wished to order a fried egg it was necessary to use an elaborate code to describe how it should be cooked (back in Newcastle, a fried egg was a fried egg full stop (sorry, period). Once, when ordering a fried egg 'easy over' in what I took to be an impeccably correct fashion, I was completely unnerved by being asked 'what would you like that on?' (it turned out that 'a plate' was not the right answer). I remember my surprise when I learned that English was not my native tongue (I evidently spoke with a 'strong' British accent) but rather the name of the language spoken (at least at that time) by the American nation. This, to me quite remarkable, perception was strengthened when a young teenager, wishing to confirm his understanding that England was 'kind of misty', surprised me by asking 'does everyone over there speak English?'. Through this there ran the same thread that causes Australians to question why their land is always put at the bottom of the globe. (Not to mention the fate of Gallileo when he suggested that Earth did not lie at the centre of the universe.) That I was at all surprised to find England as strange and as unimportant to the citizens of Indiana as Indiana was to their British counterparts, was a measure of my youthful innocence. Conversely, there was nothing innocent about the Senator from Wisconsin and the brooding presence of McCarthyism which hung over the scientific community as much as it ever did over Hollywood. Once, this even touched me in a somewhat oblique fashion. Like everyone 'on Faculty', I was called upon to swear renewed allegiance to the American Flag. Fearing the Tower of London and execution for treason, I protested that I owed my allegiance elsewhere. After long and careful thought, an indulgent university administration offered me an alternative. With a straight face I promised that I would not 'attempt to overthrow the United States Government while resident in the State of Indiana', thinking the while that if this were to become my ambition, I might do it with good conscience from neighbouring Illinois.

To a young, and then not widely travelled young man from the north of England, Indiana was a new experience in many other ways. It got dark early and abruptly (I was used to summer days when, at least in June, it never really got dark at all) but an Indiana summer had an exotic magic of its own. In the corn fields, evenings were black velvet perforated by fireflies and alive with the sound of crickets. Winters were cold. On my way to the lab I often walked through, rather than past, campus buildings in order to temper the impact,on my unprotected ears and nose, of

air much colder than I had ever before experienced. For some time I shared a room in an apartment with George Fritz. Tall, laconic and looking (I thought) like Humphrey Bogart (Figure 1), George liked to turn off the steam -heat and open the windows at night. Not daring to argue and too poor, or too mean, to buy another blanket I shivered until George departed at dawn and then, turning on the heat and closing the windows, I enjoyed an hour of blissful warmth before the 8 am start of my working day. Harry's tiny lab (Figure 1) was located in the 'Pierce Conservatory and Small Animal House'. To be precise, it opened into a glass house and was immediately above the basement which housed the small animals, a fact that became more and more obvious as the summer advanced. Pride of place was given to the refrigerated Warburg respirometer. This was run at 25 ° but for three months in the summer of 1954, given the absence of air conditioning and the proximity of the glass house, it never started the day at less than 35 °C. Back home it had been warm too. My father sent me a cutting from a newspaper recording the fact that 80 °F temperatures had driven Londoners to the coast. I responded with a local headline which read 'Cold Front from Canada brings welcome relief to heat-stricken Indiana, temperature plummets to 90 °'.

To the local populace, Purdue University was theirs; the 'Memorial Union' a favourite Sunday eating place. Slow to appreciate this, it surprised me at first when, if I happened to be in the lab on Sunday, the door would open and I would find myself in conversation with a local farmer, keen to know how I was spending *his* money. I ate breakfast in the Union, amazed to discover (being accustomed to much more limited choice) that it was possible to buy several sorts of hot and cold cereal, not to mention such outlandish things as melons. 'Hot tea' was also possible although asking for it provoked a certain amount of consternation. My evening meal was in a 'Hamburger Heaven' close to the University Apartments where I had a bowl of soup (5 cents), a Hamburger (15 cents) and a coffee (5 cents). Purdue was a 'dry' campus and 'dry' applied to the whole of West Lafayette but, once I knew the ropes, I found that I could enjoy a beer in the back room of what was euphemistically described as 'Harry's (no relation to Harry Beevers) Chocolate Shop'. Such delicacy didn't apply on the other side of the Wabash where the beer flowed freely and it was rumoured that there were no less than six brothels.

For the first time, Purdue (and, most of all, Harry) convinced me that I might aspire to scientific competence. Within a few weeks it became clear that the endosperm of Castor Bean lent itself to the isolation of mitochondria; a procedure not without its bizarre aspects. The first part had to be done in Harry's lab. Then I had to run across the railway line to use the only available refrigerated centrifuge in the main building. This was a matter of some concern to the kindly janitor, invariably taking it easy by the coke machine, who used to cry 'slow down Dave, running ain't going to get you no place'. It was better advice than I was to realize until retirement but at least the mitochondria functioned more rapidly, by a couple of orders of magnitude, than any plant mitochondria then described in the literature (Beevers and Walker 1956). They also behaved differently, in many ways, from animal mitochondria which, of course, led to the inevitable but (as it eventually turned out incorrect) suggestion that they were damaged. One thing which puzzled us at the time was the fact that, with oxaloacetate as substrate, O_2 uptake could be delayed by as much as an hour (see Figure 2) before it took off at great speed (Walker and Beevers 1956). I believe that it was a conversation that Harry had with Carl Price which finally led us to conclude that NADPH (or DPNH as it was then called), generated by the operation of the Krebs' Cycle, was being preferentially re-oxidized by the reduction of oxaloacetate to malate until establishment of a steady-state equilibrium permitted its re-oxidation by the cytochrome system and molecular oxygen. For me this was an object lesson in the dangers of not recognizing what ought to have been staring me in the face, a common enough failure in science but not one to afford much comfort as a shared experience. Lags (or induction periods) were to come back to haunt me in later life (see e.g. Walker 1973, 1976) Indeed Mordhay Avron, once introducing me in his capacity as a conference chairman, made me smile by saying that I had 'built a career on them' (see e.g. Walker 1973). Though I wasn't to know it in 1953, I would eventually read much about photosynthetic induction in Eugene Rabinowitch's remarkable book (Rabinowitch 1956). What I did do at the time, on a visit with Harry to Urbana, was to ask Rabinowitch when the final volume would appear. (Answer: 'My publisher has stopped asking me and asks only my wife') What is worse, and with a brash temerity that I can now scarcely believe, I also asked Robert Emerson if he felt that he had invested too much time in the famous quantum yield controversy with Otto Warburg. It was the sort of question which might have earned a snarl from a lesser man but, instead, there was a patient but rather weary 'yes'.

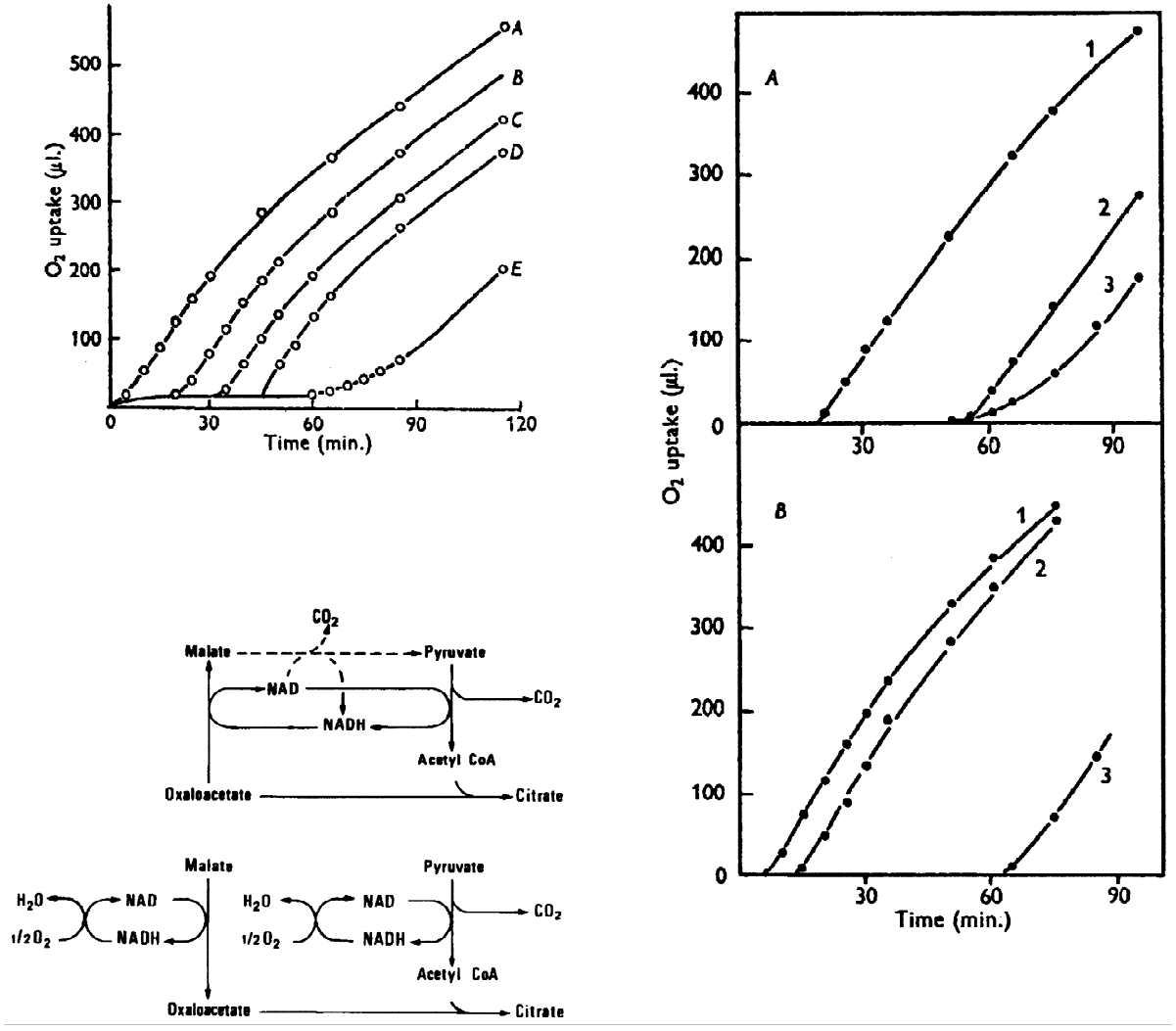


Figure 2. My first Encounter with Lags. When mitochondria isolated from *Ricinus communis* endosperm were incubated with oxaloacetate, pyruvate and one or more cofactors (Beevers and Walker 1956), the ensuing oxygen uptake (measured manometrically) started abruptly but only after a lag which could last as long as an hour (it was first observed after manometric measurements had been left to run during a seminar). *Top left:* the effect of cocarboxylase on pyruvate/oxaloacetate oxidation. Thiamine pyrophosphate (TPP) was added to 4 replicate mitochondrial suspensions at zero time (shortest lag), and thereafter at 15 minute intervals (progressively longer lags) or not at all (longest lag). *Top right:* oxaloacetate oxidation. Curve 1, control with all added cofactors. Curve 2, minus TPP. Curve 3, minus coenzyme A. *Lower right:* oxaloacetate plus pyruvate oxidation. Curve 1, control. Curve 2, minus coenzyme A. Curve 3, minus TPP. Carl Price suggested that NADH generated by pyruvate oxidation is preferentially re-oxidized in the reduction of oxaloacetate to malate by malic dehydrogenase (see *bottom left, upper*) until all of the oxaloacetate is consumed and NADH is then re-oxidized by molecular oxygen (*bottom left, lower*, after Douce 1985).

Back to Newcastle

In 1954 it was time to resume my PhD studies in an environment still cold enough to remind me of my naval days. As I have written elsewhere (Walker 1995a) of the 'Harrison Laboratory' (where my predecessors included Harry Beevers and my contemporaries Stan Ranson (Figure 1), PN (Dhani) Avadhani, John Brown, Mary Stiller and the three Bradbeer brothers, Bill, Clive and Phil):

> the lab temperature scarcely ever rose above 12 °C. Attempts to raise that a degree or two by lighting every available Bunsen burner were usually defeated by the arrival of Professor Meirion Thomas (Figure 1). What else he wore I do not know but there was a certainly a raincoat on top of an overcoat

> and beneath that a tweed suit, a pullover and a waistcoat. So garbed he would ride briskly into the University on his bicycle, stride into the lab, and, with a cry of 'stuffy in here', throw open all the windows.

Nor were biochemicals more abundant than warmth:

> No doubt there were graduate students, even then, who were able to avail themselves of the services of the Sigma Chemical Company but, in Newcastle, that was thought to be as sinful as travelling by taxi. So one morning I found myself on my way back, by bus, from the local abattoir, clutching a metal vasculum (polystyrene was unknown) containing livers, from freshly slaughtered pigs, packed in dry ice. Washing facilities were not offered to raw university students so I was obliged to ignore my blood-stained hands and act as though a similarly blood-stained container, encrusted with ice and trailing streams of vapour, like something from a horror movie, was a commonplace sight. It says much for the travelling British public of that time that no one gave it, or me, as much as a second glance. Back at the lab there were galvanized buckets of boiling water and a meat grinder. Bucket biochemistry really was bucket biochemistry and eventually yielded a few milligrams of putative TPN. Chemical reduction gave an appropriate peak at 340 nm but how to tell how much of this was NADP and not NAD? So it was back to the abattoir and to the extraction of a known, NADP-specific, isocitric dehydrogenase from pig hearts. Imagine my dismay when this enzyme failed to reduce my painfully extracted NADP. Clutching at straws, I prevailed on Stan Ranson to chromatograph the isocitrate that I had bought from a company long suspected of digging all of its products from the same mine. Sure enough, whatever else it was, it was not isocitrate. So there were more buckets and lots of *Bryophyllum/Kalanchoe* leaves. Finally, after weeks of graft, there was the immense satisfaction of putting together each laboriously won enzyme, coenzyme and substrate and watching the needle on the spectrophotometer announce that my NADP was indeed NADP.

Fortified by Purdue experiences, *Kalanchoe crenata* no longer seemed an unassailable bastion. Moreover, my Purdue experiences with oxaloacetate had reminded me that although the reaction catalyzed by malic dehydrogenase could, in the context of the Krebs' cycle, be pushed and pulled in the direction of malate oxidation, its underlying equilibrium overwhelmingly favoured reduction. This was relevant to dark accumulation of malate in Crassulacean plants. When I had started working for my PhD in 1952, Ochoa's malic enzyme (Ochoa et al. 1948) seemed like the only

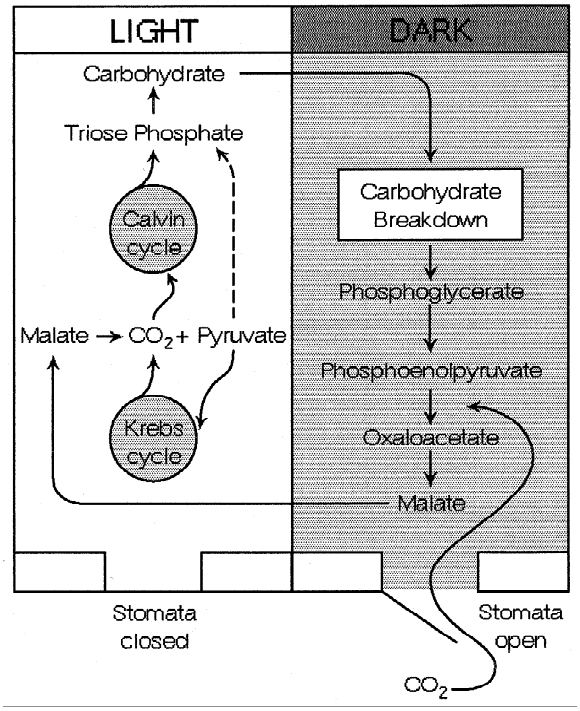


Figure 3. Crassulacean Acid Metabolism (CAM) was a term first employed by the late Meirion Thomas, in whose Newcastle laboratory much of the early work on this type of photosynthesis, including mine, was done. CAM is typical of (but not restricted to) the family Crassulaceae – hence the name. Like C4 photosynthesis, it involves preliminary fixation of CO_2 by phosphoenolpyruvate carboxylase to give oxaloacetate which is reduced to malate. Subsequently the malic acid is decarboxylated and the CO_2 re-fixed Indeed C4 photosynthesis was once flippantly referred to as 'CAM mit Kranz' (i.e. 'Kranz type anatomy in which there are two concentric layers of cells, containing chloroplasts, surrounding the vascular tissues of the leaves) and, however inadequate this description, it does remind us of the fact that, in C4 photosynthesis, preliminary fixation and refixation occur in separate compartments whereas, in CAM, these processes are separated in time. CAM is basically an adaptation to water shortage. CAM plants conserve H_2O by keeping their stomata closed by day, when high temperatures normally bring about high water loss by transpiration. Behind closed stomata, the Calvin Cycle proceeds happily in a CO_2 enriched atmosphere produced by decarboxylation of malic acid (itself resulting from carboxylation of PEP during the preceding night when the stomata were open). Many CAM plants show a degree of succulence, related to the storage of malic acid which is a necessary feature of this process. Opening of stomata by night and closure by day is the converse of the stomatal behaviour of C3 species.

feasible catalyst of this process but even then, like the Wood and Werkman reaction (Wood and Werkman 1936), or 'would't work man reaction' as we quite unreasonably dubbed it, it seemed unlikely in view of the equilibrium position of the reaction. So it came as a revelation when Bandurski and Greiner (1953) characterized phosphoenolpyruvate (PEP) carboxylase from wheat-germ. Here, at last, was the obvious candidate. Having devised a way of getting to grips with succulent leaves full of acid and short on protein, it led me to conclude that dark acidification in CAM was attributable to the combined efforts of PEPcarboxylase and malic dehydrogenase (Walker 1952) and light deacidification to malic enzyme (Figure 3 and Walker 1960, 1962, 1992a). It also led me to the nerve-racking experience of presenting a paper for the first time (at a meeting of the Society of Experimental Biology in Edinburgh). I was told, by some, that such an experience would become less terrifying with use and experience. They were wrong.

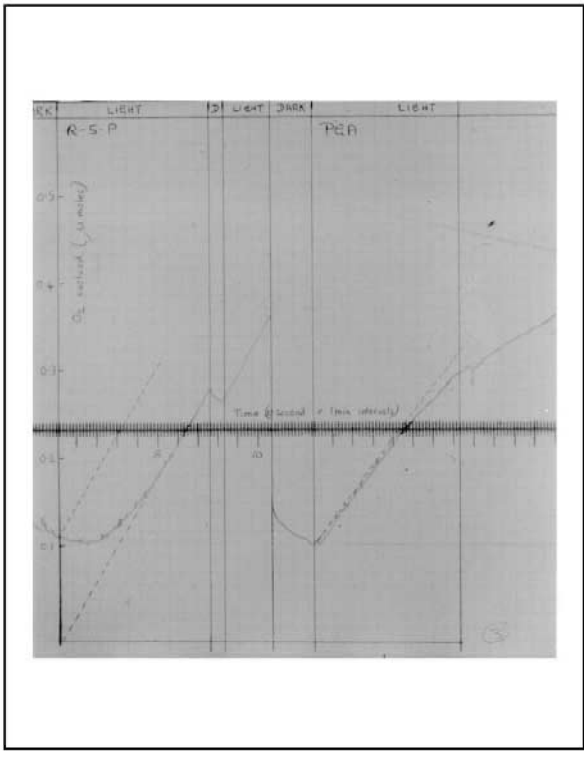
In the English system of the time, the fate of my PhD lay in the hands of a single external examiner. So this was how I was to meet Robert (Robin) Hill (Figure 4), for tea, in the unlikely surroundings of a pub called 'The Crow's Nest' in Newcastle's Haymarket. The tea was serene enough but when it was followed, back in King's College by the actual examination, Robin's initial questioning was so unexpectedly fierce that every thought of a successful outcome fled from my mind. Fortunately I was somehow able to convince him that I had a rudimentary knowledge of enzyme kinetics and the laws of thermodynamics and there followed not only a PhD but an association that was to last for more than forty years.

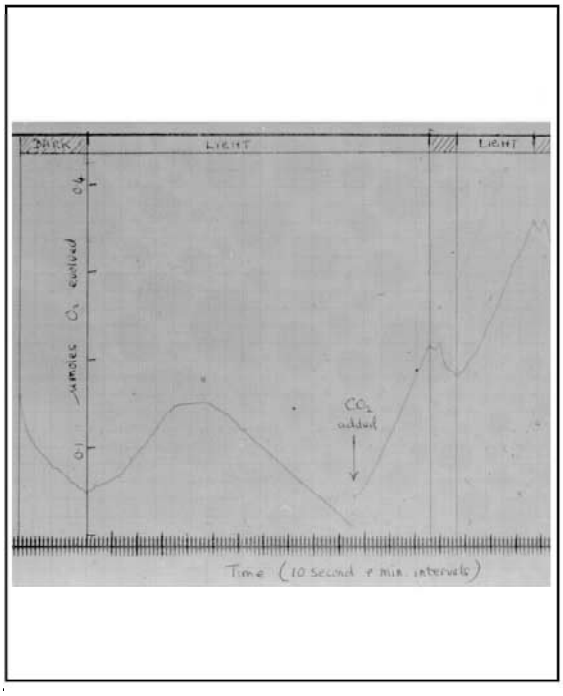
Ivory towers

What made the University of Cambridge a possibilty was an ICI (Imperial Chemical Industries) postdoctoral fellowship. These normally went to chemists. I disgraced myself at the interview by not remembering, despite having worked with them for a year at Purdue, the derivation of the word 'mitochondria' (mine looked *round*, rather than 'thread-like' under the microscope). Nevertheless, perhaps because the committee thought that it was time to give one to a botanist, I found myself with a fellowship. More importantly it was a fellowship which was equally valid in another university and therefore allowed me to accept Hill's invitation to join him in the Biochemistry Department at Cambridge. It might just as well have been Mecca. Newcastle was excellent but Cambridge Biochemistry was something else. The list of staff bristled with household names, familiar from every biochemistry textbook. A young man called Frederick Sanger, destined to become a double Nobel Laureate, worked in the lab across the corridor. The emptiness of Joseph Needham's room by the stairs empasized its owner's preoocupation with Chinese science. Robin took me to the building across the way and introduced me to David Keilin. Over in the Botany Department, F.F. Blackman still appeared from time to time and, wonder of wonders, even invited me to give a lecture. Years later I learned that, had I known who they were, I might have gaped at James Watson and Francis Crick discussing the 'double helix' in 'The Eagle' as I drank my beer at an adjacent table (a juxtaposition which put me in mind of the man 'who was in the next room when Rutherford split the atom'). I walked around Cambridge pinching myself and basking in this reflected eminence. Everyone else took it for granted. Cambridge radiated an air of timelessness and effortless superiority. Newly married, Shirley and I lived for a time in Granchester, immortalized by Rupert Brooke's poetry. The clock on the church still 'stood at ten to three' It was evident that, if Cambridge could survive two world wars, it was not to about to be overwhelmed by the possible consequences of the British and French seizure of the Suez Canal, which coincided with my arrival and brought petrol rationing in its wake.

Photophosphorylation

Robin's lab has often been described (Bendall 1945; Kamen 1992; Walker 1992b) but no description, however vivid, could ever do justice to the crazy reality. What I saw was even smaller and much darker than Harry's old lab in the Pierce Conservatory (Figure 1), teak benches, every inch covered with a plethora of tubes and bottles, a smell of alchemy and total disorder (except that Robin knew where everything was). Although I was allowed in the inner sanctum, I did not actually work there. My place was in a bay in an adjoining room which I shared for a time with Derek Bendall. It took me some time to discover what we were about. Robin was not one for telling colleagues what to work on. He strewed the path with clues until the message went home. So, in this faltering fashion, I was encouraged to start work on photophosphorylation (Walker and Hill 1958). Of course, in Berkeley it was Dan Arnon and Robin's erstwhile student Bob





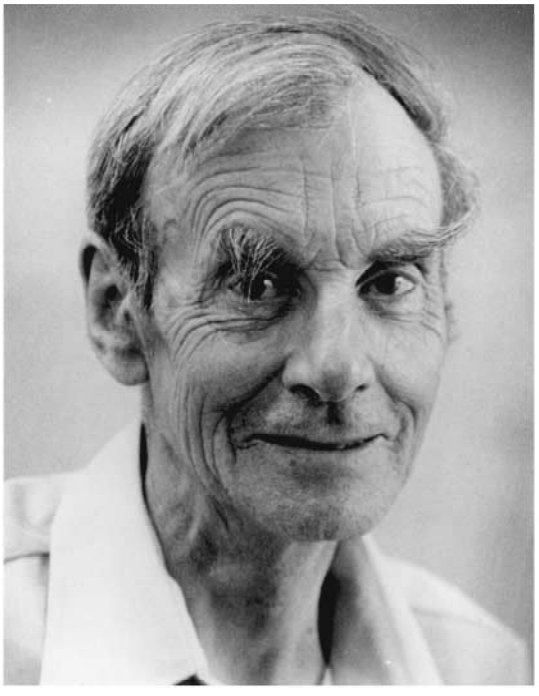


Figure 4. Top left: Charles Whittingham *Top right:* pen-recorder chart showing first ever polarographic measurements of CO_2-dependent O_2 evolution from isolated chloroplasts showing initial lag and its absence in the presence of added phosphoglycerate (Walker and Hill 1967). *Bottom left:* as for top right, showing decline in O_2 evolution in the absence of added CO_2 and renewal of O_2 evolution following the addition of CO_2. *Bottom right:* Robert (Robin) Hill.

Whatley who had been making the running for some time (see e.g. Arnon et al. 1954). Too good an act to follow for a newly fledged post-doc (Walker 1995b), although a little light was cast on the redox potentials of so-called co-factors (Walker and Hill 1958, Hill and Walker 1959) which may, in the most minor of ways, have contributed to Robin's eventual formulation of the Z-scheme. Apart from that, I learned how to make active thylakoid preparations in the proverbial Tris-NaCl and contrived to fashion an illuminated bath out of an inverted cake cover bought from the antique shop round the corner (Hill and Walker 1959). There was very little money for biochemicals, so the 3 ml reaction mixtures so beloved of the Lawrence Radiation Laboratory were decreased to 0.3 ml. Photophosphorylation was measured by molybdate blue determination of the extent of orthophosphate esterification. The reaction vessels were 10 ml centrifuge tubes. This had the virtue that experiment, subsequent centrifugation and analysis could all be carried out in the same vessel but the daily task of preparing chloroplasts, accurately hand-pipetting 7–10 additions to a final volume of 0.3 ml in a dozen or more tubes, illuminating the mixtures, stopping the reactions, and carrying out the molybdate analysis was the most exacting and dreary work imaginable. Compensation dwelt in daily communion with real genius. I once stayed for two or three weeks with the Hills at Vatches Farm in Comberton. It was Robin's custom to discuss the day's results before dinner. This might take anything from half an hour to an hour and a half. No signal, that I ever detected, was exchanged with Robin's wife Priscilla or with others of his family but, when *he* was ready, the evening's meal appeared as though by magic. I have met and admired some very accomplished scientists and often felt out of my depth. With Robin it was more than that. Intellectually, I was a small child in the presence of an adult (Walker 1992b).

'Up and down the City Road'

It was at Cambridge that I met another pillar of the British photosynthesis establishment, Charles Whittingham (Figure 4). He was soon to move to the Chair at Queen Mary College in the University of London and, when he did (in 1958), he offered me a lectureship. Given my circumstances, this offer did not fill me with the unqualified pleasure which it might have done. Lecturing has always filled me with terror; the thought of having to earn a living in this way therefore filled me with foreboding. But, by then, I had a daughter as well as a wife to support and there seemed little prospect of a job in research. My deep rooted dislike of lecturing was never to be overcome. Kind friends who told me that I lectured well only served to increase my apprehension. That apart, Queen Mary College (QMC) was very pleasant. Set in London's East End it was about as different from Cambridge as could be imagined but it was small, friendly and cheerful. I got to know Tom Delieu, then a workshop technician and soon my closest friend. With Charles Whittingham I became joint supervisor to an exceptionally bright PhD student, name of Geoffrey Hind, who very sensibly disregarded all of the advice that I was able to offer him. The pace was leisurely enough to permit occasional lunch time excursions to 'The Prospect of Whitby' where Judge Jeffries once used to watch the bodies swinging over Execution Dock. Now and again we even walked under the Thames from the Isle of Dogs to Greenwich to admire 'The Cutty Sark'. Doug Graham sang at the bench.

Having established a small lab I worked at first with Doug Graham who was just finishing his Ph.D. with Charles Whittingham, prior to emigration to Sydney. Working with Mung Bean cotyledons, all that we could grow at QMC, we proved (at least to our satisfaction) that the Kreb's Cycle continued to turn in the light despite contemporary evidence to the contrary (Graham and Walker 1962). With Roger Hiller I ventured into the strange phenomenon of exchange transamination (Hiller and Walker 1961). Using this device (Dearing and Walker 1960) and PEP carboxylase from my Newcastle days I once synthesized $20,000 worth of L-aspartate-4-C^{14} for a Californian company which promised me $100 for my trouble but forgot to enclose the cheque. Reminders proved ineffective. By chance, however, I soon had an opportunity to discuss that omission with them in person (and become instantly $100 richer) at a crowded Federation meeting in Atlantic City. By then, in 1962, I was enjoying six months leave on a Kettering fellowship with Zuni Zelitch in New Haven.

Stomata

Arriving in someone's home complete with wife and two very young children (Marney and Richard) is probably not the best way of starting a scientific collaboration particularly if you inadvertently play havoc with their plumbing within the first hour. It says everything

for the forbearance and endless hospitality of Ruth and Zuni Zelitch that they have made us equally welcome on many more occasions over the intervening years. I had come to the Connecticut Agricultural Experiment Station to learn about glycollate metabolism (sorry Ed Tolbert!) but Zuni was deep into stomata (see e.g. Zelitch and Walker 1964) and who was I to demur? Besides, Zuni is renowned as a maverick free thinker, cast in the Robert Boyle (1661) mould, happy to swim against the tide; the veritable stuff that scientific advance is made of. Even so, I agree that, when applied to stomata, the German proverb had it right. (This proclaims, courtesy of Klaus Raschke, that the deeper you look into a hole the more likely you are to fall into it.) Stomata certainly humbled me. I came to share the view of Mr Nolan, for fifty years or more in the Connecticut Agricultural Research Station, who said that they put him in mind of the rear end of an old mare that he often sat behind in his youth. The Biochemistry Department at that time (with H.B. Vickery still in command, although I rarely saw him) had an old world quality that reminded me strongly of the place in which I'd learned my elementary chemistry in Newcastle. Imposing laboratory note books were placed in a strong room each night for safety. Aprons were worn instead of lab coats, giving us the quality of a barbers' quartet. 'Havana Shade' was still a tobacco to be conjured with. Zuni's assistant, Isabel Namenworth, still departed each weekend for the cultural sanctity of New York City (beyond the borders of which it was her firm belief that civilization ceased). In the great outside world there were 'The Beatles', 'The Bay of Pigs' and the sound of 'Spring Pipers'. Arthur Miller was spotted on campus carrying a plant (seemingly in search of someone who might identify it). There was still 'a table down at Morays, wherever that might be'. I lifted my daughter on to my shoulders to see John. F. Kennedy go past in a cavalcade all too soon to be recalled by the tragic events in Dallas, Texas.

Intact chloroplasts

Back in London, Charles Whittingham (Figure 4) changed my life for ever by getting a grant for work on the isolation of fully functional chloroplasts and proposing that I should do the actual work. Not only that, he had established a field laboratory at Dytchleys, the estate of a dower house now used by the college to provide sports facilities for its students. At that time, my family and I lived in Epping, separated by seventeen miles and Epping Forest from London's East End. Dytchleys was about the same distance to the south of Epping but driving there was to follow an incredibly rural route, the more remarkable that it was within 20 miles of Lloyds' of London and The Bank of England. In those idyllic surroundings, with the help (one half day a week) of a young technician called Carl Baldry, I was to start the isolation of chloroplasts which I hoped would one day fix CO_2 as well as the intact leaf. In those days the going rate was about 2 μmoles. CO_2 mg^{-1} chlorophyll$\cdot h^{-1}$. By the time that I had jacked this up by an order of magnitude it seemed worth a brief publication (Walker 1964) even though there was still another order of magnitude to go. Naturally, before embarking on this endeavour I had asked Hill's advice and it was he who suggested third molar sugar as the osmoticum. Wary of glucose (or other metabolically active sugars which work just as well) I chose sorbitol. It seemed like a good idea to use orthophosphate as a buffer, to include a little Mg^{++} and Mn^{++} for the sake of electron transport, enough chelating agent to keep these in solution in the presence of orthophosphate and some ascorbate to stop things becoming adversely oxidized (Walker 1971a). At that time, only isoascorbate was available as the sodium salt from UK suppliers, a choice which was to cause confusion in other lands. At the time it was not known that the chloroplast *in vivo* was surrounded by a double envelope which enclosed the stroma (Walker 1967, 1970a) and that what many regarded as intact isolated chloroplasts were nothing more than stacked thylakoids, capable of electron transport and photophosphorylation but essentially devoid of the stromal enzymes required for CO_2 assimilation. In retrospect it is clear that the chloroplasts which Hill used in his classic experiments in the 1930s would have fixed measurable quantities of $^{14}CO_2$ if that isotope of carbon had then been available to test that possibility and that mine would have done much better if I had known how best to treat them once isolated. However, so far as I was concerned, other events were to intervene. By now I was a 'Reader' a promotion which doubled my salary at a stroke. For the first time, at least by my standards, I was relatively affluent. Living (in a beautiful house) on the outskirts of London in the swinging sixties, happily married, my research at Dytchleys going well, what more could anyone ask? The answer, had I known it, was more of the same but that was not to be. Charles Whittingham moved to Imperial College, 'my' funding and my access to Dytchleys moved with him.

After many weeks of uncertainty I met my new head of department for the first time. He told me that he would not wish to have a member of his staff working 20 miles off campus at Dytchleys. (A pronouncement which, as Shirley said at the time, was the equivalent to taking candy from a baby.) This was an awful blow but at least it put an end to protracted indecision. Driving away from that meeting I can still remember the waves of relief that flowed over me. Whatever else I might do with my life it would have to involve moving from QMC. This decision was re-enforced by the Granting Agency. It told me that, while I might well command financial support in my own right, it would not (having already awarded a grant for work on isolated chloroplasts) be prepared to offer me another for precisely the same purpose. Happily, salvation was at hand. Charles Whittingham was prepared to take me with him to Imperial College where I was offered the post of Reader in Enzymology.

Chloroplasts in envelopes

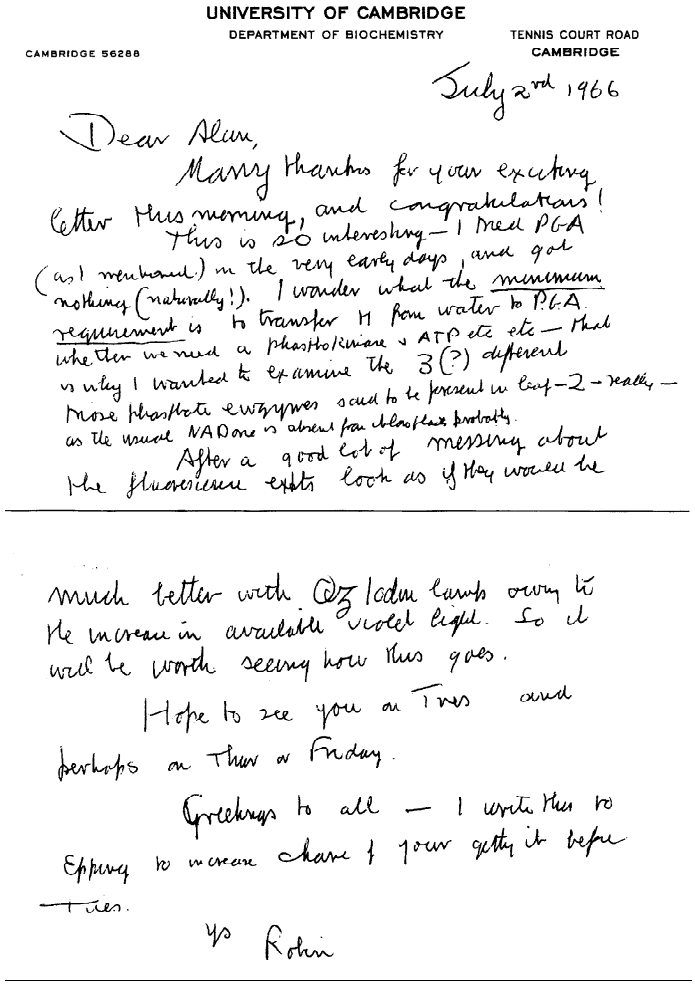
Much as I was glad to make the move to Imperial College, the seeds were already being sown for a further move. Queen Mary College was homely. Dytchleys was twenty miles drive through pleasant countryside. The Imperial College for Science and Technology was large, austere, and three hours a day (commuting by tube and bus) from Epping. Accordingly, it was not long before I wished to escape from London altogether. In the meantime there were compensations. I found money to offer the late Carl Baldry full time work as an assistant experimental officer and recruited Chris Bucke and, after him, Bill Cockburn as my post-docs. Whatever we were individually, we made a formidable team. I still find it hard to understand how we managed to do so much work in a day. Experimental design was a joint endeavour. Every working procedure at the bench was split three ways. At the most crucial moments no one spoke, each did whatever was required without hesitation. Everything worked. It was pure delight.

Inevitably, intact chloroplasts continued to be a major pre-occupation. Until this period 'intactness' itself had been an ill-defined concept but we were, with the help of Dennis Greenwood's distinguished electron microscopy, able to establish that those isolated chloroplasts which retained the ability to fix CO_2 did so because they still possessed their stroma within a bounding membrane or envelope that was double in nature (Walker 1967). Since intactness and function went hand in hand we continued our quest for better and better rates. Our target was a 100 μmoles $O_2 \cdot mg \cdot chlorophyll^{-1} . h^{-1}$ (i.e. the same order of magnitude as that sustained by the parent tissue) but, by the mid 1960s, our studies on photosynthetic induction had also focused our attention on what might, and what might not, readily cross the chloroplast envelope. No doubt these preoccupations were in my mind when, for the 1968 Photosynthesis Congress in Freudenstadt, I wrote:

> we have occasionally been asked why we, why in fact anyone, should have spent a considerable time trying to isolate chloroplasts which would assimilate carbon at rates approaching those of the intact plant. I believe that there are several very good reasons which are perhaps self-evident. One, however, which is immediately relevant to the present discussion is that if chloroplast can perform as well as the intact plant then it is reasonable to suppose that its envelope may have retained at least some of its normal characteristics. I believe that the notion that the isolated chloroplast leaks freely should be regarded with suspicion, if not rejected entirely. *Certainly some molecules pass easily in and out of the chloroplast but it is becoming increasingly evident that the properties of the envelope are such that there is some measure, perhaps a very large measure, of selectivity and control* (Walker 1969).

Disappointment and elation

Before the 1968 Photosynthesis Congress, just as we reached the magic 100 (Bucke et al. 1966), it was difficult to avoid some feeling of chagrin when we learned that we had been pipped at the post by Jensen and Bassham (1966) who made headlines in the New York Times with similarly high rates of CO_2 fixation. Moreover, their high rates were achieved without the requirement for 'catalytic' sugar phosphates which we used as a matter of routine to diminish the initial induction period before the attainment of maximum rate. With characteristic generosity, Dick Jensen acknowledged that his preparations had been based on procedures virtually identical to ours but they differed in one crucial aspect (the use of inorganic pyrophosphate) which, as subsequent events where to show (see e.g. Walker 1974), was to have immensely important implications in regard to movements of molecules in and out of the chloroplast. In the meantime, however,



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July 2nd 1966

Dear Alan,

Many thanks for your exciting letter this morning, and congratulations! This is so interesting — I read PGA (as I mentioned) in the very early days, and got nothing (naturally!). I wonder what the minimum requirement is to transfer H from water to PGA. whether we need a phosphokinase + ATP etc etc — that is why I wanted to examine the 3(?) different those phosphate enzymes said to be present in leaf — really — as the usual NADPase is absent from chloroplast protocols.

After a good bit of messing about the fluorescence exists look as if they would be much better with Cz lodine lamps owing to the increase in available violet light. So it will be worth seeing how this goes.

I hope to see you on Tues and perhaps on Thurs or Friday.

Greetings to all — I write this to Epping to increase chance of your getting it before Tues.

Yrs Robin

Figure 5. Robin Hill's response to my letter telling him of the results illustrated by the charts pictured in Figure 4.

there was to be one of those rare moments guaranteed to send a young researcher rushing into the street crying 'Eureka'. It started with weekly journeys to Cambridge; to visit Robin Hill in the Department of Biochemistry in Tennis Court Road. Coming to Imperial College from my home in Epping by public transport was not a pleasure. Driving in the opposite direction, through rural Essex, was as welcome a relief as my lost commuting to Dytchleys. Entering Cambridge always lifted my spirits. I was in search of oxygen. Until this time, all of my work with intact chloroplasts had been with $^{14}CO_2$ and, although everything suggested normal function, there remained the nasty possibility that ascorbate in the reaction mixtures might be serving as the reductant. Moreover, the only previously reported observation of CO_2-dependent O_2 evolution by isolated chloroplasts was of rates so low that they could be detected only with difficulty and in oxygen-free conditions (Allen et al. 1955). However even these were to be envied because although I prepared chloroplasts from pea leaves carried from Imperial to Cambridge they displayed not the slightest tendency to evolve O_2 whereas those prepared by my colleagues at Imperial, from identical leaves, fixed $^{14}CO_2$ at good rates. Two things changed this in spectacular fashion. Robin introduced me to the Clark electrode (1956) and I was so impressed by its obvious potential that I acquired a vibrating glass O_2 electrode to use in my own laboratory. By the time it arrived I had found it possible to buy spinach from Covent Garden and to persuade the Chelsea Physic Garden to grow it for my use. The first time that I put spinach chloroplasts in the electrode vessel and started illumination I was rewarded by a change in the slope of the trace on the pen recorder which pro-

gressed through what, by then, we had come to regard as a characteristic induction period, into full-blown oxygen evolution (Figures 4 and 5). Scarcely able to believe that the missing O_2 had been found, I set up a second mixture to which I added 3-phosphoglycerate at the moment of illumination, believing as I did that this should abolish the lag. Watching the immediate evolution which followed (Walker and Hill 1967) engendered feelings which must surely have matched those that propelled Archimedes from his bath. Two mysteries remain to this day. Fully functional chloroplasts can sometimes be prepared from spinach left languishing in the dark for a month on a cold-room floor. Preparing chloroplasts from anything other than newly harvested pea shoots is a near impossibility. Secondly the O_2 evolution consequent upon the addition of 3-phosphoglycerate (PGA) ought, in principle, be that associated with the re-oxidation of NADP reduced by electrons (plus hydrogen ions) (equivalent to hydrogen atoms from water. In intact chloroplasts it is not quite that simple because O_2 evolution promoted by PGA quickly declines unless CO_2 is also present (Stokes and Walker 1972). In other words, in intact chloroplasts, but not in a reconstituted chloroplast system (Stokes and Walker 1971), it is still mostly CO_2-dependent O_2 evolution which is stimulated by PGA rather than PGA-dependent O_2 evolution. There is still no good explanation why internally generated PGA should be different from externally added PGA.

CO_2-dependent O_2 evolution – the requirement for orthophosphate

Continuous recording of O_2 evolution made our lives a great deal more simple. Until that moment our measurements had been largely based on incorporation of radioactivity from $^{14}CO_2$. Now, when necessary (Figure 6), we could follow both O_2 evolution and CO_2 uptake simultaneously (Walker et al. 1968) and, for many purposes, rely on O_2 evolution alone for evaluating kinetics. Accordingly, we were soon well aware that molecules like NADP, ferricyanide and ribulose 1,5-bisphosphate (RuBP) seemed not to cross the intact envelope at detectable rates, that adenylates moved across very slowly, ribose 5-phosphate (R5P) more quickly and orthophosphate, PGA and dihydroxyacetone phosphate (DHAP) very rapidly indeed (see e.g., Walker 1969, 1974). One of our most satisfying and important observations was that, in order to maintain CO_2-dependent O_2 evolution, it was necessary to add orthophosphate (Cockburn et al. 1967) and that there was then a stoichiometry of three molecules of O_2 evolved for every molecule of orthophosphate added (Figure 6). We had never detected sucrose amongst the products of fixation and it seemed clear that, if the immediate products of carbon assimilation, were triose phosphates (and the like) then there must be appropriate importation of orthophosphate rather than internal recycling. Similarly, it became evident at an early stage that there was an optimal concentration of external orthophosphate, that larger concentrations were inhibitory (Figure 6), that orthophosphate inhibition was ameliorated by inorganic pyrophosphate and reversed, with appropriate kinetics, by carbon cycle intermediates which crossed the chloroplast envelope (see e.g. Walker 1974). Accordingly, when I started, in 1969, to write an article on photosynthesis with Tony Crofts (which had been commissioned, in the way of these things, for publication in Annual Reviews of Biochemistry the following summer) I wrote

> *if sugar phosphates are exported from the chloroplast there must be a corresponding import of phosphate (in some form) if steady state photosynthesis is to be maintained*

and

> *the concept of specific permeases in chloroplasts must be seriously examined. A direct obligatory exchange between orthophosphate (outside) and sugar phosphate (inside) could account for the inhibition of photosynthesis by orthophosphate and its reversal by sugar phosphate.* (Walker and Crofts 1970 and Figure 6)

Early in 1970, when Hans Heldt visited Imperial College, he and I discussed this proposition. We agreed that, in view of his experience and his recent evaluation of adenylate movement in chloroplasts, it would be appropriate for him to use centrifugal filtration to test such a hypothesis and that PGA/orthophosphate exchange would be an obvious starting point. This he and his colleagues did to such good purpose (Heldt and Rapley 1970) that our 'obligatory exchange' became the 'phosphate translocator'. Theory became reality. Naturally, we rejoiced in this consummate verification of our proposal but, human nature being what it is, I must confess that there have been times since when I have felt that acknowledgement, in some quarters, of the contribution of Carl Baldry, Chris Bucke, Bill Cockburn and myself to this story, might have been a shade more generous. Heldt's elegant and painstaking characterization of the phosphate translocator (see e.g. Heldt 1976) is not in question. Nor is the fact that he

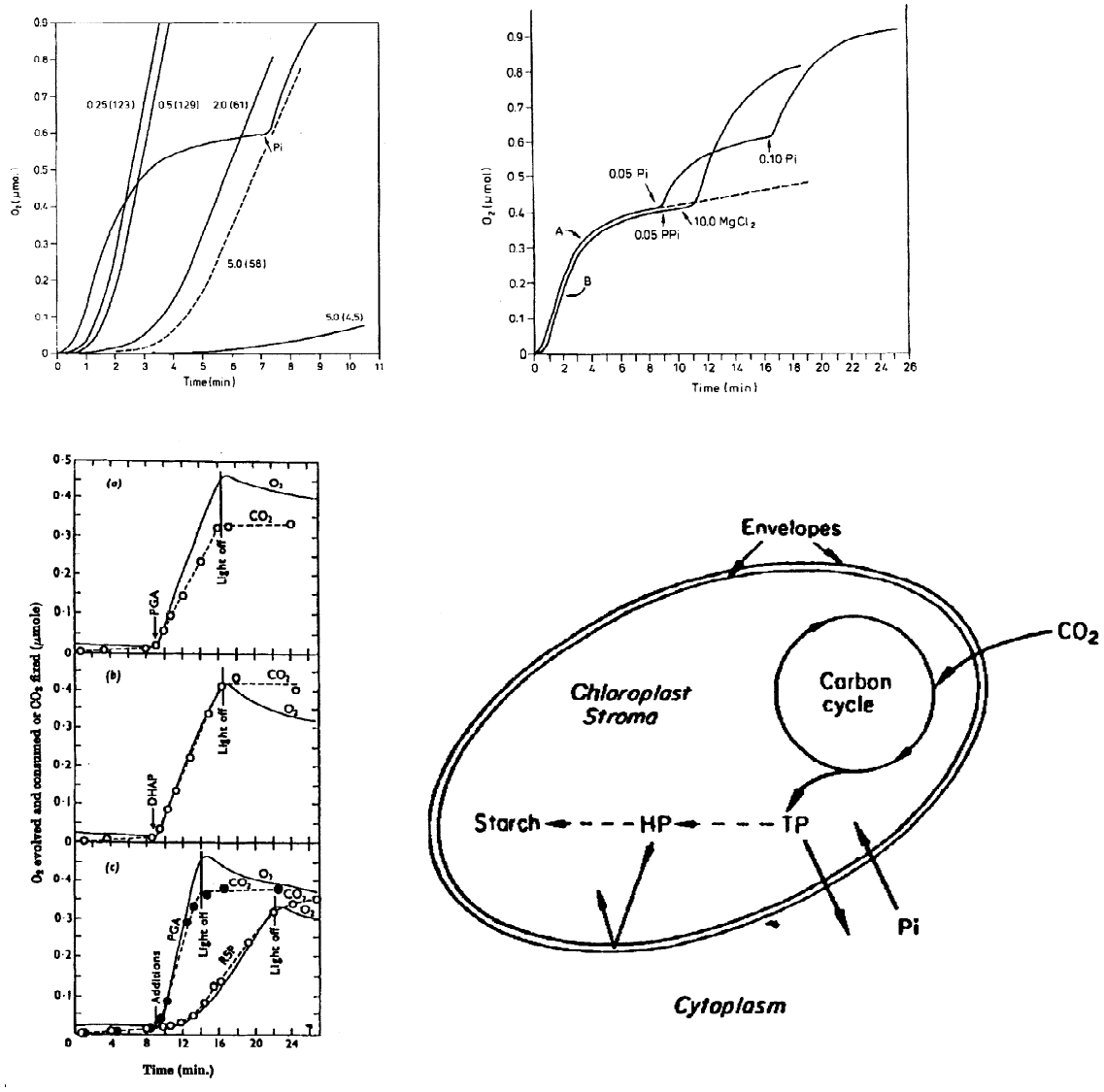

Figure 6. Data of the sort which showed that, for optimal photosynthesis, isolated chloroplasts had to be supplied with external orthophosphate (Pi) within a narrow concentration band. *Top left:* time course of photosynthesis with increasing amounts of Pi (from zero, left, in which O_2 evolution soon slowed for lack of Pi, to 5.0 mm Pi, right, in which the initial induction period was extended into marked inhibition). *Top right:* 3 to 1 stoichiometry between O_2 evolved and Pi added, plus 6 to 1 stoichiometry between O_2 evolved and inorganic (PPi) added (in accord with the relationship $3\ CO_2 + 3\ H_2O + 1\ Pi \rightarrow 1$ triose phosphate $+ 3\ O_2$). *Bottom left:* simultaneous measurement of O_2 and CO_2 showing reversal of Pi inhibition by added sugar phosphates such as 3-phosphoglycerate (PGA) dihydroxyacetonephosphate (DHAP) and ribose 5-phosphate (R5P). Such results (see text), together with the lack of sucrose synthesis by chloroplasts, led to the formulation of proposals, e.g. *bottom right:* concerning the exchange of metabolites between stroma and cytosol and suggestions that there must be obligatory exchange between external Pi and internal sugar phosphates.

and his colleagues were to make this field their own and to advance it immensely. At the same time there is no doubt in my mind (nor, I believe, has Hans ever suggested otherwise) that the phosphate translocator was '*discovered*' at Imperial College, and subsequently '*characterized*' in Munich and Goettingen.

'With eyes severe and beard of formal cut'

Science, like any other human activity is pursued at a price. Behind the closed doors of my laboratory, working with friends and colleagues there was warmth and the excitement of discovery; the daily stimulation that

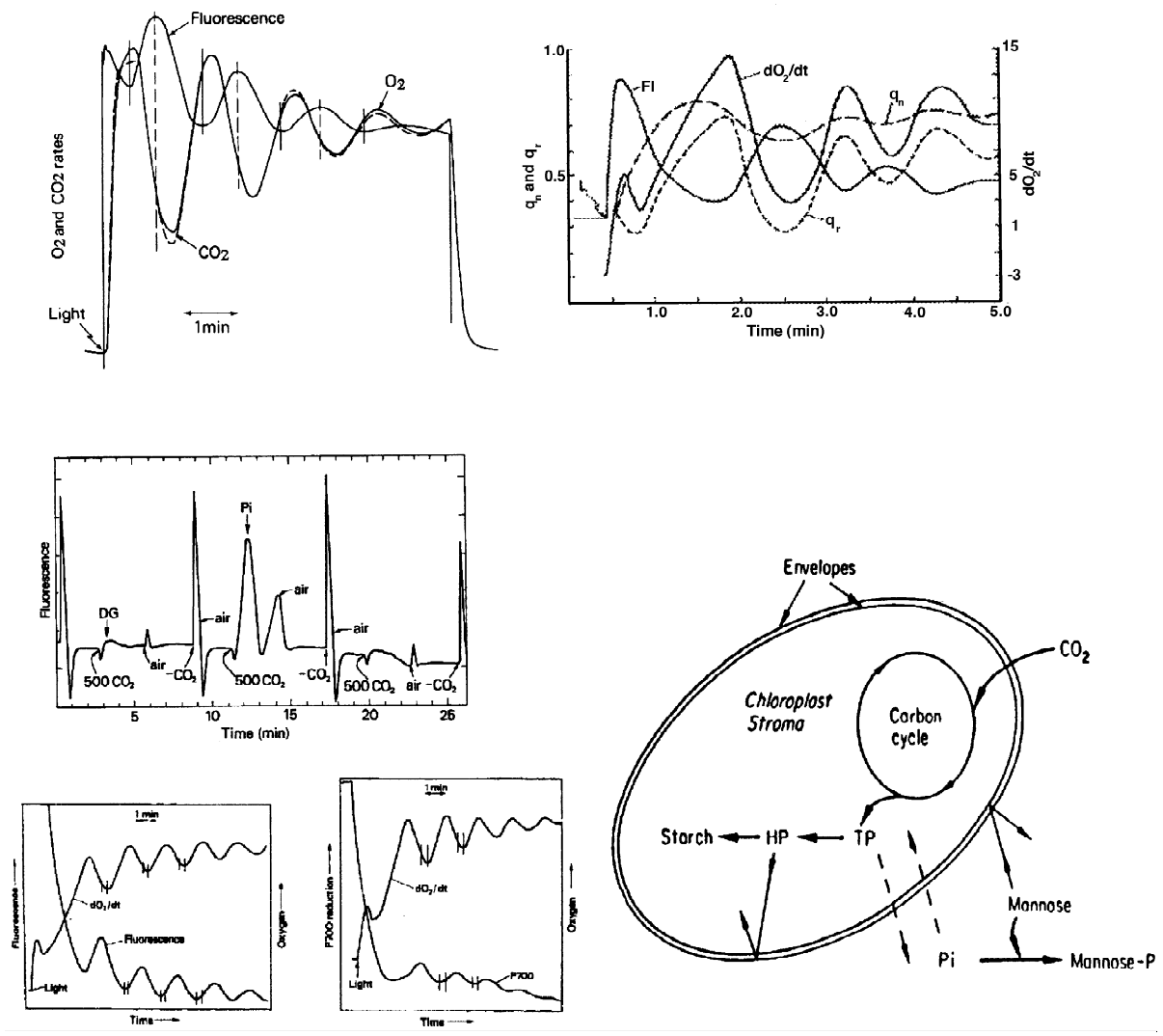

Figure 7. Oscillations in photosynthesis in leaves. *Top left:* anti-parallel relationship between chlorophyll *a* fluorescence emission on the one hand and O_2 evolution and CO_2 uptake on the other. *Top right:* similar anti-parallel relationship between O_2 evolution and fluorescence but also showing how these gas exchange signals relate to q_P and q_N. The former (q_P) is a measure (coefficient) of quenching (i.e. suppression), by photochemical events, of chlorophyll *a* fluorescence emission and an indicator of the oxidation status of the primary electron acceptor (Q_A), in Photosystem II. The latter (q_N) is a measure of the corresponding quenching of fluorescence by non-photochemical events of which energy quenching (q_e) is a large component which has been used as an indicator of the hydrogen ion concentration across the thylakoid membrane (ΔpH). *Bottom left:* similar relationships between O_2 evolution, fluorescence emission and the oxidation status of the primary electron acceptor associated with the (chlorophyll reaction center) pigment (P_{700}) which absorbs light at a wavelength of 700 nm in Photosystem I, in addition to that in the blue end of the spectrum (for a fuller discussion see Havaux et al. 1991; Walker 1992c). Oscillations of this nature can be induced by abrupt increases in CO_2 concentration, abrupt illumination, etc. (Walker 1992). The magnitude of ensuing oscillations is influenced by the availability of inorganic phosphate (Pi) being greatest when cytosolic Pi is limiting (e.g. in high light, high CO_2 etc.). *Bottom right:* this illustrates how an artificial limitation on Pi availability can be imposed by feeding Pi sequestering agents, such as D-mannose or 2-deoxyglucose (DG) which are converted to the corresponding phosphates in the cytosol so that less Pi is available to re-enter the chloroplasts. In turn, this limits triose phosphate (TP) export and enhances starch synthesis via hexose phosphate (HP). The effect on fluorescence is seen, *centre right:* in a sequence in which oscillations were induced by a succession of abrupt changes in $[CO_2]$. The first increase in $[CO_2]$ from air to 500 ppm CO_2 had little effect but, within 8 minutes of the start of DG feeding a second increase in $[CO_2]$ from air to 500 ppm CO_2 was followed by pronounced oscillations. Conversely these oscillation were dampened by Pi feeding at the time indicated. Such a sequence can be repeated indefinitely (see e.g. Sivak and Walker 1987).

came from scientific problems identified and solved. For much of the time I could imagine no better life. Then, as I approached my fortieth birthday I experienced what it was fashionable at the time to describe as 'a mid-life crisis'. Until then there had been direction, certainties and targets. Set, as it were on rails, school had been followed by navy, university, first degree, second degree, post-doc, marriage, first child, lectureship, second child, first house, second house, readership. Perhaps, in all of this, no time to contemplate purpose or lack of it. Then, quite suddenly, a need to ask why. Finding no satisfactory answer, all that suggested itself as a palliative was escape from those aspects of my life which I liked least. These were readily identified. First and foremost there was Imperial College itself. Queen Mary College had been small and friendly. Immediate friends and colleagues apart, Imperial College (also ostensibly part of the same University of London) was just the opposite, the most awful and soulless academic establishment that I have ever encountered. Travel, to and from my home in Epping, came a close second in disgust. It took three hours in total, two out of the three on the London underground. The only redeeming feature was that, on the inward journey (since Epping was at the end of the line), I could get a corner seat and there, in miserable discomfort, I wrote twenty or so papers encompassing what I still regard as my most important work. So I left home at 7am and returned at 8pm, rarely seeing my young children except at weekends when, inevitably, their first joy was to be taken back to the pleasures of the big city. Anxious to be home, I walked past the people standing in line outside the Albert Hall waiting to attend concerts and, apart from the visual pleasures of the King's Road (at the height of the mini-skirt era) and the sounds of 'The Beatles' and 'The Stones', I might as well have spent 'the swinging sixties' in John of Groats as in London. It became my greatest ambition to leave London. The remedy was obvious but not so easily achieved. It was 1970 before I was offered the Chair of Biology in the University of Sheffield. Life immediately improved. There is much to be said for running away from problems. Travel became negligible. We bought a huge and immensely comfortable Victorian stone-built house (with no less than five cellars and fifteen fire-places) for the current price of a modest motor vehicle. My student Judy Emett and David Stokes (the first of a succession of splendid Australian post-docs) had moved from London with me and Ross Lilley, Jens Schwenn, Toni Slabas and Simon Robinson were soon to follow when he left. Alice Herold and Hwa Chen became my research students. My technician Krystyna Holborow (nee Kosciukiewicz) was to keep my lab in first-class order for years to come. My early visitors included Gerry Edwards with whom I wrote 'C3 C4' (Edwards and Walker 1983). My research flourished although I was never able to get adequate funding to exploit what I regarded as the vast potential of the reconstituted chloroplast system (Stokes et al. 1972).

Australia

Having been scientifically weaned on phosphoenolpyruvate carboxylase and Crassulacean Acid Metabolism (CAM), the story that was emerging in the 1960s, first from Hugo Kortschak (at that time I wasn't aware of the Russian observations) and then from Hal Hatch and Roger Slack, was immensely interesting to me but, like many, I was baffled by what purpose might be served in photosynthesis by a preliminary incorporation of CO_2 into C4 acids (those containing 4 carbons) followed by transcarboxylation into phosphoglycerate. This led to a long correspondence with Hal Hatch, to an invitation (almost as soon as I had arrived in Sheffield) to attend the 1970 conference on C4 photosynthesis at the Australian National University (ANU) in Canberra (where I was to find myself cast in the role of skeptic) and to life-long friendships with the principal protagonists. The elucidation of C4 photosynthesis was, and is, a masterpiece of plant biochemistry but to bystanders like myself, until 1970, a mass of confusing contradiction. The Canberra meeting altered all that (Walker 1971b). The magnitude and importance of what had already been achieved by Hal Hatch, Roger Slack and their colleagues became apparent. Everything started to fall into place. There were 'i's to dot and 't's to cross but C4 photosynthesis had become of age. At a purely personal level, Australia itself was also a revelation. There can be few more beautiful campuses than that of the ANU to which Barry Osmond and Hal Hatch (Figure 8) so kindly invited me so often. Shirley and I made many new friends and renewed many old friendships. The smell of the Eucalypts, the sound of Australian birds, the wine, the oysters at Bateman's Bay are treasures that will stay with me for always. Even cycling 10 miles each way to work through Adelaide in 40 °C temperatures was more pleasure than pain and I have often regretted failing to grasp two earlier opportunities which would have kept me in Australia on a permanent basis. As it was, my visits (mainly to ANU

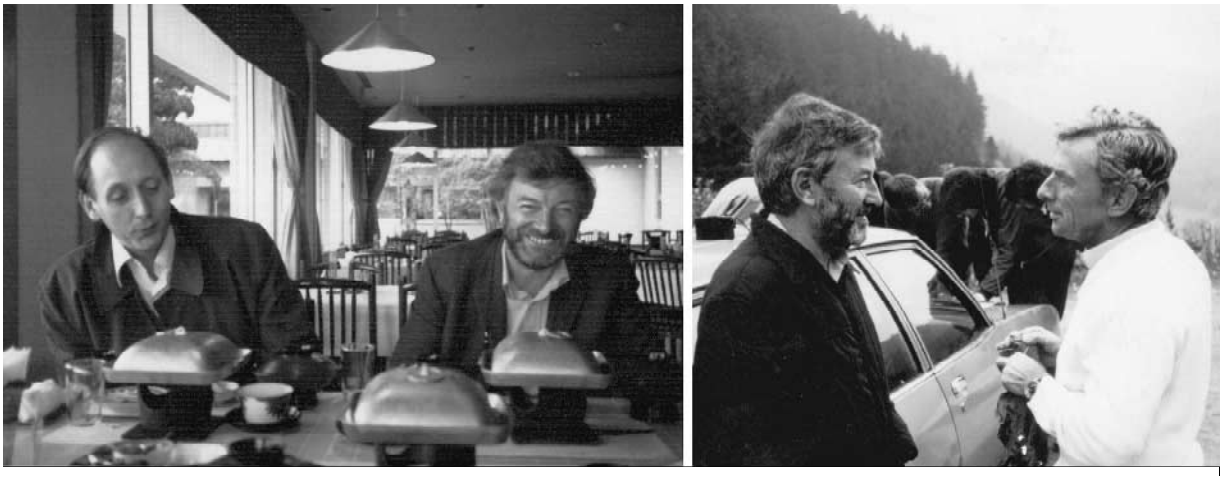

Figure 8. Left: with Barry Osmond. *Right:* with Hal Hatch.

Canberra but also to renew happy co-operation with Simon Robinson in John Possingham's laboratory in Adelaide) were always too short, although on one visit to Barry Osmond's laboratory, prompted by Christa Critchley and discussions with Jan Anderson, I did fulfil a long-standing ambition to start (Walker 1981) an examination of the relationship between chlorophyll *a* fluorescence and photosynthetic carbon assimilation. Whatever impact this, and the closely related phenomenon of oscillations (Figure 7 and Walker 1992c,d) had on photosynthesis in general, it certainly influenced the future direction of my own work and a quick scan through my publication list now reveals some 35 papers since 1981 which have the word 'fluorescence' in the title. Though logic and monumental work from many laboratories (for reviews see e.g Krause and Weis 1991; and Govindjee 1995) tells me otherwise, I still find it remarkable that photosynthetic rates can be accurately evaluated from fluorescence data (cf. Walker and Seaton 1992) or that feeding phosphate (or phosphate sequestering agents) through leaf petioles (see e.g. Sivak and Walker 1985) can dramatically alter fluorescence emission in a matter of minutes (Figure 7).

Biddlestone

In 1968 I was admitted to University College Hospital for an excruciatingly painful and unpleasant operation which was to rid me of the first of three afflictions which have cast shadows over my life and inevitably, I suppose, made me the person, and therefore the scientist, that I am. My 'mad' aunt, now in her nineties, told me recently that I will never be able to change the world. Naturally she is right (particularly if you take a cosmic view of things) but surely scientists must believe that they can do just that, at least in some collective fashion, and perhaps there are those amongst us, like myself, who are driven by dissatisfaction with their lot like the proverbial artist in the garret. Certainly migraines, which came to me in my 35th year and left as abruptly in my 65th, dominated my life and changed the way that I looked at everything. But I digress: back in 1968, faced with surgery that was most unlikely to be life threatening, I nevertheless thought that I was about to die. When I recovered conciousness and found that I was still alive but in the most awful pain, all that I could think to do was to concentrate my mind on happier things. In consequence, I prevailed upon my father-in law (Bill Mason, to whom I owe much in addition to giving me his only child, Shirley, in matrimony) to rent a cottage in the country. I knew the very spot and that was how we came to rent 'The Priest's House' in a village of six houses. We were there for ten years, and being on the spot, eventually able to buy a derelict cottage, now refurbished, which we still have. Biddlestone became a refuge, a hiding place from the more unpleasant aspects of academic life and, in the words of my daughter Marney, the best thing that I have ever done. By now, we have been there 28 years.

Figure 9. Prototype 'Delieu Walker' (DW) apparatus for polarographic measurement of oxygen by isolated chloroplasts etc. *Above:* glass chamber with plunger to exclude air and plexiglass water jacket. *Below:* electrode disc removed to show central platinum cathode, membrane and magnetic follower (flea).

Oxygen electrodes and such

As a child, learning to swim, I had not at first opened my eyes under water, more fearful perhaps of injury to my sight than drowning. When I realized that it was safe to do so it was, of course, a revelation. When Robin Hill introduced me to the Clark electrode (Clark 1956) it was much the same. The ability to undertake continuous measurement; to see immediately the consequences of experimental intervention is immensely liberating. Sadly, however, the apparatus then available was far from ideal. Following Ohmar Khayam's edict, Tom Delieu and I therefore sought to 'crush this sorry scheme of things entire and remould it nearer to the heart's desire'. I made pencil drawings on the backs of envelopes. Tom, a master craftsman, turned these into physical reality (Figure 9). It would be nice to think that we solved all of the problems. We did not. Clark's electrode was a major achievement. We simply put it into a mechanical environment better suited to our purpose (Delieu and Walker 1972) and the world still awaits a device which will perform well in all circumstances. Even so there was soon more demand for our apparatus than we could meet ourselves. Derek Bendall introduced me to John Humby, a manufacturer in search of a product. We had a need for someone to make us apparatus. So started a long and mutually beneficial co-operation. Some years later, despairing of the availability of cheap and simple apparatus for measurement of photosynthesis by students, I asked Tom to make me a chamber which would accommodate a Clark-type electrode and a leaf-disc *in the gas phase* (Delieu and Walker 1981; Walker 1987). To my surprise, it soon became clear that this crude device had potential as a serious research instrument. Computer programming by John McCauly and George Seaton and the combined efforts of Dave Thoday, Richard Poole and many others at 'Hansatech Instruments' worked wonders. Measurements of quantum yield which once took giants of the world of photosynthesis many hours to perform could be done in as many minutes. So far as my own research was concerned, the facility to make simultaneous continuous recording of rates of O_2 evolution and chlorophyll *a* fluorescence emission, combined with a growing interest in oscillations (which cemented lasting friendship and co-operation with Agu Laisk) made it immediately apparent that that there was an anti-parallel relationship between these two signals (see e.g. Walker 1992a,b)

Germany

In September 1939, when Neville Chamberlain made his momentous broadcast ('a state of war now exists...') I was eleven and, in the way of small boys, simply excited rather than horrified. Mercifully the bombs that soon fell frequently around my home hurt no-one that I knew personally and my age insulated me from coming face to face with less immediate death and

Figure 10. Ulrich Heber, myself and Robin Hill discussing oscillations, or whatever, over beer.

mutilation. Even so, the war was real enough and near enough to leave me in no doubt that Germans were my enemy. Had someone told me then that, within a few years, many Germans would become my closest friends I would have found it very difficult to believe. But so it turned out. For this, for the manner in which he has greatly influenced my life, my science and my pleasure in the driest of dry Sylvaner, I owe much to Ulrich Heber (Figure 10) whom I met for the first time at the Aberystwyth meeting (see Walker 1967) in 1965. An invitation to visit him in Dusseldorf followed and I have been spending time in German laboratories ever since; mostly with Ulrich Heber in Würzburg and another good friend, Erwin Latzko, in Münster. Consequentially there have been many purely social visits, (Oh for the company of Ute and Kumar Mukherjee and Pinkus Müller beer to gladden the heart!) and reciprocal exchanges which have widened to include many younger colleagues. Thus I take great pleasure in the small part that I have played in initiating this aspect of Anglo-German co-operation, not least because it tells me that *there really is* a forward-looking and open-minded international scientific community.

David Hall's flying circus

In 1970, David Hall put together the first of an unlikely (it would be unkind to say 'motley') group of 'instructors', drawn from many nations, whose aim it was to fly to the far corners of the world (Madurai, in Southern India, was first on the list) to share their combined knowledge of photosynthesis with anyone who might wish to share it. Time has blurred the composition of the very first group but it certainly included Harry Bolhar-Nordenkampf (desperately ill with food poisoning), Gerry Edwards (as unflappable as ever, joining me in singing the Sheffield Carols), 'Hassa' Egneus (delivered into raptures by the delights of India's Southern cuisine) and Peter Lea (anxious to remind me that photosynthesis had as much to do with N as with C). Then, or in later years, there were Miguel Guerrero and Avigad Vonshak (in whose joint company I drank a great deal of unfamiliar alcohol), Geoffrey Hind and Richard Leegood (totally unmoved by local laboratory disasters and as relaxed as they would have been in Brookhaven or Sheffield), Minno Reporter (unceremoniously 'dumped', like me, by ocean waves on a Brazilian beach) the ubiquitous Jonathan Scurlock keeping everything in order to David Hall's satisfaction and many more. Last, but far from least, were unbelievably hospitable hosts, caring for our every need from China to Brazil, from Thailand to Barbados and splendid, patient and resilient 'students' from everywhere imaginable. Altogether, this was teaching of the very best and most satisfying sort – students who wished to know, unpaid instructors who worked extremely hard to overcome all of the difficulties involved in doing practical instruction in strange laboratories and unfamiliar circumstances. We all had problems of every conceivable sort. Once we had survived the vagaries of strange airports and their associated officials, there were wasps in the electric sockets, fluctuations in the electric supply which would have shamed a yo-yo, cobras on the foot-paths, an absence of what would be regarded in more favoured places as commonplace reagents, ubiquitous mosquitoes and a perennial shortage of bottled water, or the like, which could quench thirst without penalty. All this and more, regarded with equanimity by David Hall on whose back rested the awesome overall responsibility for everything. As an academic experience and an exercise in international understanding and goodwill it would be difficult to imagine its equal.

'The sixth age'

The year 1979 was very kind to me and it seems like a good point at which to quit this history. Given that I am writing in 1996, that might seem a shade premature but 17 years for reflection can't be bad and, who knows,

some fate might grant me another 17 years of retirement to ponder the rest. Moreover, younger readers, at least, might find interest in a scientific world that they have not experienced themselves, one not so dominated by molecular biology and computing, one already imbued with flavours of alchemy, pre-electronic balances, dubious pH meters, Warburg respirometers and out-moded techniques. There are other reasons. Honesty about long past events might be excusable, even forgivable. Honesty about more recent events can be hurtful. In addition, 1979 brought a change of government to Britain and a lady called Margaret Thatcher who was as well regarded abroad, and as much disliked at home, as her Russian counterpart Michael Gorbachov. He put an end to the Soviet Union, she put an end to much of what people like myself liked best about England, not least the best aspects of its university system. I came very close to leaving academic work entirely at this time but, instead, took refuge in an offer which allowed me to establish a 'Research Group for Photosynthesis' which grew and flourished and metamorphosed into 'The Robert Hill Institute'. Thanks to colleagues, staff, students and visitors too many to mention, to Hill himself who maintained a paternal interest to the end, and more support from the University of Sheffield than could have been reasonably expected, this acquired the enviable reputation that it continues to enjoy today under the guidance of Peter Horton (Chairman) and Richard Leegood. All of this proved very rewarding but, at the purely personal level, there was more pain than pleasure, particularly in my last ten years. My closest friend, Tom Delieu, died and my own health deteriorated as events pushed me into an administrative role for which I was singularly unqualified and ill-suited. My many mistakes were harshly judged and not readily forgiven, my actions and motives rarely understood. Acting (as I thought and hoped) in the best interests of all I naturally pleased very few. At a wider level, government support for science ebbed away. Mass unemployment brought a divided society. Lawlessness and self-interest flourished where market forces ruled. These were to be my unhappiest professional years.

'That ends this strange eventful history'

But I would not wish to finish on a sad or sour note and, again at a purely personal level, that is readily rectified. As a boy I thought longingly of the sea-going apprenticeship which, even before his sixteenth birthday, took my father to the far corners of the earth. However, in pursuit of my profession I eventually finished up in many more countries than he did, sometimes as a resident, always amongst colleagues and friends. This has meant much more than simple pleasure and satisfaction. To date, retirement has been pure bliss and I have a legacy of friends and memories of a sort that do not often flow from otherwise comparable professions. This is a century in which there have been many changes for the good. It has also been one in which the greatest atrocities have been perpetrated, atrocities fashioned out of religous bigotry and irrational prejudice. Clearly international science is not above and beyond human frailty but at least it is based on a degree of rationality and knowledge which recognizes no borders. As such, it often seems to engender some degree of humility amongst its practitioners, perhaps knowing as they must, that they are at least as likely to be as wrong the next man and that there is no way of repealing the laws of thermodynamics. Whatever the reasons, being a member of the photosynthesis community has been an immensely rewarding experience

Acknowledgements

In addition to the endless help that I have received from my scientific friends and colleagues, many (but sadly not all) of whom it has been possible to identify by name in this article (and without whom none of this would have been possible), I am immensely grateful to the unfaltering support from my family. Most recently I am very grateful to Govindjee, not only for giving me this opportunity but also for his encouragement and forbearance during its preparation.

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