

Norman Alan Walker 1929–2013

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Alan Walker (he never used ‘Norman’) developed pioneering electrophysiological methods to make major contributions to our understanding of mechanisms and energetics of transport of solutes across plant cell membranes. He used giant cells of characean algae to measure membrane electrical potential differences and conductances that were combined with measurements of fluxes of inorganic ions and organic nutrients obtained with radioactive tracers. Alan built up a strong research group at the University of Sydney and had many external collaborations that considerably widened the scope of his research in relation to membrane biology of characean cells and well beyond. His considerable skills in mathematical modelling contributed significantly to research led by others on plant-fungal (mycorrhizal) symbiosis, movement of solutes through the cytoplasmic connections between plant cells (plasmodesmata), the membrane transport mechanisms that regulate the release of nutrients from coats of developing legume seeds, and even nutrient cycling in Arctic ecosystems. Alan had strong political views and definite aesthetic tastes that included aspects of visual arts (ceramics and impressionist paintings) and music. He was very interested in good food and wine, and other interests included vertebrate palaeontology and bird-watching. He had many friends with similar tastes, who share fond memories of him.

Early Years, Family and Education

Norman Alan Walker was born in Brisbane on 12 December 1929, son of Frederick Walker and his wife Clarissa (née Drane). He always hated the name Norman and refused to use it. Frederick (Fred) was a bank worker and the family moved to several small towns in rural Queensland, where Alan attended a succession of primary schools. Fred became bank manager at Jandowae in the Darling Downs, near the border with New South Wales, when Alan was ten years old and his brother Geoffrey (Geof) eight. Alan was frustrated by his early education because he could not get enough books to satisfy his active mind. Between 1943 and 1946 he was a boarder at Towoomba Grammar School, and left as Dux of the school.

In 1947, Alan won a John Black scholarship and University Open Scholarship to the University of Queensland, and graduated BSc, with First Class Honours in Physics in 1953. In the same year he married Rona Holt, an artist, also from Queensland. He then held a CSIRO postgraduate studentship at the University of Tasmania, and studied for his PhD in the Physics



Department under the supervision of Professor A. L. McAulay, who was a pioneer biophysicist in Australia. During this time Alan himself became a pioneer in the use of microelectrodes

for measuring electrical potential differences (PDs) across plant cell membranes. He used *Nitella*, one of the characean algae that have giant cylindrical internodal cells. These algae had already been used to some extent in Germany as model plants for study of plant ionic relations. They have since provided a wealth of data on ion transport, cytoplasmic streaming and cell-to-cell solute transport, but became unpopular in the molecular biology era, as the genome is very large and has still not been sequenced. Alan overcame problems that do not occur with animal cells: the presence of cell walls in plant cells and the thinness of the peripheral cytoplasm compared with the very large central vacuoles. His first published paper gave separate values for the PDs across the plasma membrane and the tonoplast that separates cytoplasm and vacuole, and focused on the response of the PD to external potassium ions.¹

The next paper included measurements of membrane electrical resistance.² It was challenged by the formidable Professor G. E. Briggs FRS, Professor in the Cambridge Botany School, who believed in ‘free space’, i.e. relatively unrestricted penetration of inorganic ions from external media into plant cell cytoplasm. Alan’s reply convinced Briggs that the plasma membrane had a pivotal role in determining the ionic relations, as Briggs acknowledged in an addendum to that paper.³ The PhD was awarded in 1959, by which time Alan had taken up a position in CSIRO. The title of his thesis was ‘Electrical Studies on the Membranes of *Nitella*’.

Early Career: Australia and England

Between 1955 and 1964 Alan was a Research Officer in the Division of Plant Industry, CSIRO Canberra, where his two daughters (Mandy and Jude) were born in 1957 and 1959 respectively. He also spent a period in the important Physiological Laboratory at the University of Cambridge (1960–2). During this time he started biophysical experiments, some with Alex Hope (another CSIRO employee) that focused on the electrical properties of *Chara australis*, including an investigation of the relationship between the PD and the membrane permeabilities of potassium (K^+) and sodium (Na^+) ions. At the time these cations were considered to be mainly responsible for determining the PD, as in animal

cell plasma membranes. These were pioneering investigations into a very complex subject. Ion transport across the plasma membrane of Characeae (and probably of higher plants) is now known to exist in several states, each state dominated by a different population of the ions that are transported. The establishment of a particular state depends greatly on external conditions. A state dominated by K^+ uptake (now known to be through membrane channels) was found by Hope and Walker in very low external Ca^{2+} concentration and/or high K^+ concentration.⁴

For the next three years (1964–7) Alan was a Senior Research Associate in the School of Biological Sciences, University of East Anglia, in the influential plant biophysics group led by Professor Jack Dainty, who had previously spent a sabbatical in Australia and had worked with Alan and also Alex Hope. The latter also joined the group, as did another Australian, Geoff Findlay, and it established close contacts with the research group in the Cambridge University Botany School led by Enid MacRobbie (later FRS and Professor), who had been Dainty’s PhD student at the University of Edinburgh. The biophysical experiments continued, using media that were more natural (low K^+ and higher Ca^{2+} concentrations) than those used previously. It was found that ionic conductances due to fluxes of K^+ , Na^+ and chloride (Cl^-), measured using radioisotopes, were much less than the electrical conductance. The possibility that fluxes of protons (H^+) were involved was discounted.⁵ This work was completed and published soon after Alan returned to Australia. However, it was not long before he accepted the importance of active efflux of H^+ from plant cells (the ‘proton pump’).

Return to Australia: Ongoing Networking and Development of the Sydney Plant Biophysics Group

Alan returned with his family to Australia in 1967 to become Associate Professor (Biology) at the School of Biological Sciences, University of Sydney, where later he was appointed Challis Professor of Biology (1985). The research networking that had originated in England was re-established for some time. It included Hope and Findlay, who had taken up positions at Flinders University, Adelaide, and Andrew

Smith, formerly Enid MacRobbie's PhD student (and now FAA), who had arrived at the University of Adelaide. Andrew replaced Michael Pitman (later FAA), who had moved to Sydney, and was also a member of the team. The research involved studies of the biophysics of both freshwater and marine algae with giant cells, using measurements of electrical parameters of membranes and measurement of ionic fluxes using radioisotopes for Cl^- , K^+ and Na^+ , which were the main ions that determined the osmotic potential, at least in vacuoles, in these algae.⁶ The measurements on marine algae initiated an important enquiry into fluxes and electrophysiology of the chlorophyte branch of the evolutionary tree. These experiments were later continued by Alan's Postdoctoral Fellows Mary Bisson and Mary Beilby, who went on to develop successful careers at the State University of New York, Buffalo and the University of New South Wales, respectively.

A major output of this phase of the research was the book, *The Physiology of Giant Algal Cells*, written with Alex Hope, in which the influence of Alan and his colleagues can be followed.⁷ This book was written soon after the beginning of the 'Mitchellian' phase of plant membrane physiology that involved recognition of the role of proton (H^+) transport in powering transport of other ions across plant cell membranes. This recognition led to a broader appreciation that ion transport in plant cells does not closely follow the models previously extrapolated from animal cells. This conceptual breakthrough arose from the work of Peter Mitchell (later FRS and Nobel Laureate) that established the fundamental importance of H^+ transport in microorganisms, mitochondria and chloroplasts and—at first controversially—overturned past beliefs about the mechanism of synthesis of adenosine triphosphate (ATP).

Alan soon established a strong research group at the University of Sydney. This resulted mainly from his logical and charismatic teaching in cell biology and especially in biophysics, which was then by no means commonly taught in plant-based courses. He highlighted Mitchell's theory, and there was opposition for some years from those staff in the University who clung to the notion of chemical coupling to explain ATP synthesis, despite increasing evidence that this was invalid. Alan attracted a succession of

exceptional research students. As a result there were many publications, some of which are cited later (see Bibliography available online as Supplementary Material). The large biophysics group in the School of Physics, University of New South Wales, later provided several post-doctoral Fellows with physics background: Mary Beilby, Derek Laver and John Smith.

The Australian research network, known to some members of the grant-giving Australian Research Grants Committee as 'the plant membrane transport Mafia', faded away but Alan maintained very strong links with Andrew Smith, who had been an early convert to Mitchellian thinking, especially in regards to the need for active H^+ extrusion (the proton pump) and its possible involvement in the mechanism of active Cl^- transport in characean algae. Alan's background in physics became very important at this stage. As well as his mathematical and modelling skills he approached membrane transport from an energetic point of view: is it 'uphill' or 'downhill'? If it is 'uphill', how is it powered and where does the energy come from? To what extent is the proton motive force that originates from the proton pump responsible for driving transport of other ions?

The proton motive force across a membrane can be expressed as an electrochemical ion gradient that involves both the pH difference and the membrane PD. Alan had been a pioneer in measuring PDs and he needed measurements of intracellular pH to calculate proton motive force. Using *Chara australis*, he and Andrew Smith made the first chemical measurements of the pH in the cytoplasm of a plant cell.⁸ The measurements suggested that a two-proton ATPase in the plasma membrane drives the proton pump there.⁹ Later modelling and measurement of very negative membrane PDs suggest $1\text{H}^+/\text{ATP}$ stoichiometry. However, this aspect of the pump is still not fully resolved and the stoichiometry may be variable and depend on external conditions and abiotic stress.

Having addressed the energising mechanism at the plasma membrane, Alan and Andrew investigated uptake of nutrients, again using *Chara* as a model. The uptake of ammonium ions (NH_4^+) and ammonia (NH_3) was studied in collaboration with Mary Beilby, then a new post-doctoral researcher in Alan's laboratory. This work combined two techniques: measurement

of electric currents and PDs across the plasma membrane and the use of ^{14}C -labelled methylammonium (CH_3NH_3^+) as a radioactive analogue for NH_4^+ and similarly for ammonia.¹⁰ The work quantified the kinetics of NH_4^+ uptake, showing that transport involves high-affinity uniporter (i.e. not coupled to H^+ uptake), decreasing with increasing solution pH, where permeability of the free base NH_3 (and CH_3NH_2 , when present) increased. The shape of the uptake versus external concentration of the amine ions was influenced by the unstirred layers of solution around the large cylindrical cells and consequent diffusion-limitation of ions when they are rapidly absorbed by high-affinity transporters (as with NH_4^+). This finding led to a more general appraisal of the complications of unstirred solution layers, which became important in later research into Cl^- uptake and the kinetics of CO_2 uptake in photosynthesis by aquatic plants.¹¹

Chloride is an important osmoticum in many plant cells and its influx into the electronegative cell interior requires metabolic energy. Smith and Walker had earlier suggested symport with two protons per Cl^- ion.¹² Beilby and Walker found the expected depolarising current when they voltage-clamped the *Chara* plasma membrane in Cl^- -free medium and then exposed the cell to different concentrations of Cl^- . The symporter showed a low K_m and consideration of unstirred layers was important in modelling its kinetics.¹³

Following work by Bill Lucas, one of Andrew's PhD students, Alan and Andrew became interested in another aspect of the long characean cells: pH banding. Many of the freshwater Characeae inhabit alkaline ponds, where dissolved inorganic carbon (DIC) appears mainly as bicarbonate ion (HCO_3^-) rather than CO_2 . While lipophilic CO_2 and H_2CO_3 permeate easily through the membrane as uncharged molecules, HCO_3^- needs to be transported, possibly with H^+ . Characean cells activate the proton pump and H^+/OH^- channels on alternating patches encircling the cell. In the acid bands, CO_2 and H_2CO_3 become the main DIC species and enter the cell, leading to photosynthesis with high quantum yield. In alkaline bands cells excrete OH^- , which is a by-product from HCO_3^- conversion to CO_2 that enters the chloroplasts. Alan and Andrew measured external circulating currents between the bands and

formulated models for carbon uptake.¹⁴ Post-doctoral Research Fellow Mary Bisson exposed *Chara* to pH levels 9–12 and discovered that the cells enter another membrane state, where H^+/OH^- channels dominate.¹⁵ This finding was crucial for formulating models for the pH banding and carbon acquisition. The mechanisms of pH banding are still subject to research. The electrophysiological motif of localised acid and alkaline zones extends to some aquatic angiosperms, pollen tubes and roots of land plants.

All the work summarised so far used individual characean internodal cells, isolated from the long chains of cells in the intact algae. With his PhD student Thor Bostrom, Alan made a quantitative study of intercellular transport between adjacent internodal cells, and showed rapid diffusion and exchange of solutes through the cytoplasmic connections (plasmodesmata) that link these cells. The rate of exchange was limited by the rate of cytoplasmic streaming in the cells.¹⁶ These experiments highlighted another aspect of characean plants as excellent experimental system.

Yet another development in this very productive period involved a technique for perfusing *Chara* cells, involving removal of the native vacuole and cytoplasm and exposing the inner side of the plasma membrane to artificial media. With PhD student Paul Smith, Alan was able to probe the kinetics of the proton pump and its dependence on ATP and ADP concentrations. They were also the first to observe the current/voltage (I/V) characteristics of the large conductance K^+ channels in the plasma membrane.¹⁷ Further research into properties of these channels was later continued by Mary Beilby and Mark Tester.

As a result of Alan's friendship with Andrew Smith and Andrew's wife Sally (also now FAA), who researched arbuscular mycorrhizas, a very widespread soil fungal-plant symbiosis, Alan used his mathematical skills and interest in modelling to develop a novel quantitative model for the spread of arbuscular mycorrhizal (AM) fungi along growing plant roots. At this time there was considerable international interest in obtaining detailed information on the factors that influence the way in which both AM fungi and pathogenic fungi colonize plant roots, in particular the rate at which the fungi initiate colonization and the rate of fungal growth within the root cortex, all

in a growing root system. Because AM fungi do not damage the roots longer-term experiments are possible than with fungal pathogens. Sally's research in Adelaide was based on very time-consuming microscopic measurements, the only methods then available. She had collected a large amount of data on the number and length of colonization units formed by AM fungi in roots of clover of different ages and under different soil conditions. She believed that more could be made of the data and needed a collaborator to help. Alan developed equations by which the existing data could be analysed and methods of treating the outputs statistically.¹⁸

In 1981, Alan was awarded the University of Melbourne's Syme Prize for excellence in research, jointly with Andrew Smith. Alan was elected to the Australian Academy of Science in 1982. His nominator, Professor Sir Rutherford (Bob) Robertson FAA, FRS, commented that his work was characterized by a rigorous quantitative approach that was rare in plant cell physiology, and that as a result his influence was very great both nationally in Australia and internationally, which indeed it was. Alan certainly initiated new research directions, both experimental and theoretical, that were later continued by his PhD students, Postdoctoral Fellows and others.

Expanding Research Achievements until Retirement

Alan was determined to learn more about the energizing of plant membrane transport and he became interested in the adenylate concentrations in *Chara* cytoplasm. His PhD student Rob Reid (now Associate Professor at the University of Adelaide) used the firefly luciferase method to determine concentrations of ATP, ADP and AMP. They were able to check the effects (on ATP concentration) of some commonly used metabolic inhibitors. A direct correlation between cytoplasmic concentration of ATP and speed of cytoplasmic streaming provided an inbuilt 'meter' of ATP status.¹⁹ The knowledge of ATP status was also applied to study of dynamics of Cl^- influx.²⁰

With the coming of Postdoctoral Fellow John Smith, Alan revisited the earlier research topic of membrane electrical conductance, using *Chara* cells exposed to a range of external ionic conditions. The complexity of different membrane

states, both temporal and spatial, was now better understood. John Smith brought his expertise in complex impedance measurement. They recorded different conductances in acid and alkaline zones. Importantly, they also found that these patchy conditions persist even at low pH or with buffers in the external medium.²¹ In an escalation of experimental complexity, Andrew Smith joined in the experiments to measure K^+ fluxes using the radioactive tracer ^{42}K . The results suggested that membrane permeability to potassium (P_K) remains constant, while membrane electrical conductance becomes dominated by K^+ conductance only at higher external K^+ concentrations (the K^+ state of the membrane, as investigated earlier with Alex Hope⁴). Rather unexpectedly, P_K was found to decrease in darkness, and with some inhibitors and lanthanum.²²

With PhD students Kate Fairley and Peter Ryan, Alan explored the properties of amine transporters. The same porter transported increasingly substituted amines (methyl-, ethyl-, isopropyl- and dimethyl-) in their cationic form, exhibiting a Michaelis-Menten relationship.²³ Ryan and Walker investigated accumulation of NH_4^+ in the acid *Chara* vacuole (up to 70 mM). To preserve electroneutrality, the cells exported Na^+ and K^+ and imported Cl^- . In the absence of Cl^- the cells accumulated malate, which commonly occurs in vacuoles of high plants. They also looked at the metabolic processes involved in amine assimilation and related biochemistry.²⁴ With PhD student Mark Wilson, Alan became interested in urea as another source of nitrogen. The results were more complex than expected. They found three modes of transport: passive diffusion (through the lipid bilayer and possibly through channels that transport water: aquaporins) and high- and low-affinity transport systems.²⁵ The high-affinity system is an electrogenic symport with Na^+ , while the low-affinity transporter is not electrogenic.²⁶

The inner plant cell membrane, the tonoplast, is difficult to access even in large characean cells. The discovery by Lühring²⁷ that cytoplasmic droplets could be prepared enabled such access. These are produced by cutting cells in medium similar to vacuolar sap. The droplets are bound by the tonoplast and can be patch-clamped in both drop-attached and excised configurations. With PhD students Derek Laver and Kate

Fairley, Alan explored the K^+ channel in different K^+ and Ca^{2+} media on both sides of the membrane. They formulated a detailed model of the channel: diffusion-limited, activated and also blocked by Ca^{2+} (in different sites and with different Ca concentrations). Na^+ asymmetrically blocked the channel in a PD-dependent manner. The last publication appeared in 1997.²⁸ Laver went on to work on K^+ channels in animal systems when he set up his group at the University of Newcastle, New South Wales.

The potassium content of cytoplasm and vacuole is high. How do the plants maintain it in very low external concentrations? Andrew Smith and Alan starved *Chara* cells of K^+ and found electrogenic transport in the presence of Na^+ .²⁹ The elegant tracer influx and clamp current experiments suggested symport of K^+ and Na^+ with 1:1 stoichiometry. Further experiments, performed with PhD student Stephen McCulloch, were difficult, as the transport saturated quickly with rising Na^+ concentration in the cytoplasm. This problem was overcome by using cytoplasm-enriched fragments prepared by Mary Beilby. The Na^+/K^+ transporter was then characterized and modelled.³⁰ An interesting evolutionary aspect of this transporter is the limited occurrence in aquatic angiosperms, but not in land plants, where H^+ appears to be the driver ion.³¹

In parallel with the ongoing biophysical research in Sydney, Alan continued his collaboration with Sally Smith. In the 1980s, Bernard Tinker's research group at Rothamsted, England, were also addressing the details of AM fungal colonization, but using slightly different mathematical approaches. Alan went to Rothamsted to hold discussions with Tinker and collaborators. Alan felt that this exchange of ideas certainly influenced the thinking of that group and it also led to a workshop on modelling mycorrhizal colonization that was part of the program of the 6th North American Conference on Mycorrhizae (the premier international mycorrhizal conference at that time). The initial work, using existing data, was followed by jointly designed experiments to answer specific questions relating to the susceptibility of roots to colonization as they matured (are root tips more or less susceptible than older regions?), the effects of environmental conditions such as light and phosphate supply (known to influence the overall outcome in terms

of the extent of root infection) on the separate processes of infection and fungal growth within roots. Alan also played a large part in setting this modelling work in a broader plant pathological context. The work determined effective dimensions of the rhizosphere. (How far can hyphae grow through soil to infect roots? How does compression of soil as roots grow through it affect estimates of rhizosphere width?) Several more papers were published, the last in 1992.³²

The research into mycorrhizas was supported by the Australian Research Grants Committee (as was Alan's research with Andrew) and the applications were written during summer holidays on Kangaroo Island, with grant-writing in the mornings, using Alan's new 'laptop' computer (actually about as big as a current desktop), and excursions in the afternoons. The roads there were then very bad and on one visit it took Alan three days to work up the courage to switch on the computer and see whether the shaking had destroyed it: luckily it had not.

Ongoing Research Interests after Retirement

After Alan retired from the University of Sydney in 1994 he continued a collaboration with Robyn Overall (University of Sydney) that had begun in 1990 on the electrical properties of the cytoplasmic channels (plasmodesmata) that connect plant cells and can open or close in response to intracellular signals. The work was done by their PhD student Terena Holdaway-Clarke, and the last paper was published in 2001.³³ Alan also continued for some time his research interactions in Adelaide, where he had been an active member of the Centre for Plant Membrane Biology, a joint initiative involving the University of Adelaide and Flinders University, with members across Australia. He was for a short time an Adjunct Professor at the University of Adelaide (1995–8). Alan contemplated moving to Adelaide but did not do so.

From 1996 until his death in 2013 Alan held an appointment as Visiting Professor (Biophysics) in Mary Beilby's laboratory in the School of Physics at the University of New South Wales. He continued to be very productive. Alan and Mary formulated the first parameterized model to deconstruct the total I/V characteristics of the plasma membrane (or both plasma

and tonoplast membranes in series).³⁴ The modelling enables researchers to follow responses to various transporters to abiotic stress and to observe synergy among different types of transporters. This technique is still used by Mary Beilby and her PhD student Sabah Al Khazaaly and continues to deliver useful data.

Alan became actively involved in supervision of Sabah Al Khazaaly. During research into salt-induced pathology of *Chara*, which is salt-sensitive, Mary Beilby and her Postdoctoral Fellow Virginia Shepherd observed random noise in membrane PD as the cell was exposed to saline conditions. Alan at first refused to take this seriously, but once he was convinced, he helped Sabah to build a noise filter and to perform Fourier analysis.³⁵ The source of the noise is thought to arise from transient cooperative opening of H^+/OH^- channels (participating in the pH banding in unstressed Characeae). With prolonged exposure to saline conditions, these channels become activated permanently. Without a proton motive force the cell cannot withstand salinity and so these channels may play a role in salinity pathology in this plant.

While Mary Beilby was writing up her PhD thesis on the characean action potential in the late 1970s, she found the book by Hope and Walker extremely useful. With Alan now becoming part of her group, she was inspired to write a sequel. Alan suggested collaboration with characean systematics expert Michelle Casanova. Alan was initially to be a co-author but increasingly bad health intervened. Sadly, the book (*The Physiology of Characean Cells*) came out a few months after his death and is dedicated to him.³⁶

Shortly before he retired, Alan had commenced collaboration with John Patrick (University of Newcastle, New South Wales) to discover the underlying membrane transport mechanisms that regulate the release of nutrients from coats of developing legume seeds. This release fuels growth of the enclosed embryos. Wen-Hao Zhang, formerly a PhD student of Steve Tyerman (later FAA), who had also been supervised by Alan in Sydney, was appointed, as a Postdoctoral Fellow. Previous studies by John had identified the transfer cells and established that: (i) sucrose and potassium are the major transported nutrients; (ii) hydrostatic pressures (turgor) of the seed coats regulate rates of nutrient release; and (iii) sucrose release

is energy-coupled. The collaboration was characterized by robust questioning by Alan to formulate hypotheses, with John designing experiments to evaluate them. Modelling transport data led Alan to identify a novel candidate transport mechanism in which sucrose release is coupled to protons returning into the cell, following their outward transport by a proton pump.³⁷ Similarly real-time measurements of perturbations in cell hydrostatic pressures and sucrose transport provided persuasive evidence for coordinated regulation of phloem transport with membrane transport of sucrose (and other nutrients) in developing seeds by a turgor-homeostat mechanism.³⁸ Knowing the identity of the transfer cells within the seed coats allowed development of procedures to obtain enriched isolates of their protoplasts for patch-clamp studies to elucidate mechanisms governing ion release. Steve Tyerman joined the program in 1996 and most of the subsequent research was conducted in his laboratory. The ensuing publications provided unique insights into the biophysical properties and regulation of ion channels committed to releasing ions from plant cells. In some cases, the cells investigated exhibit a distinctive wall labyrinth that greatly amplifies their plasma membrane surface areas. These studies were a major breakthrough in describing the ion transport properties of these 'transfer cells'.³⁹

Alan broadened his research interests even further by collaborating with Professor R. L. (Bob) Jefferies at the University of Toronto, who had developed research interests in salt-marsh ecology when he was a Research Fellow in the University of East Anglia in the 1960s. Bob went on to research soil microbial ecology in the Canadian Arctic. Alan used his expertise in modelling to help examine the processes that influence nitrogen movement in grazed arctic ecosystems, from the cyanophytes (N_2 fixers) to the geese that graze on the swards.⁴⁰ An article published in 2010 included Alan as an author, and also Jack Dainty, who had moved on from East Anglia via Davis, California, to be Professor in Toronto from 1972 until his retirement in 1984.⁴¹ Sadly, both Bob Jefferies and Jack Dainty died before the paper appeared in print. It was also the last paper to which Alan made contributions.

Given these activities, Alan's many friendships in Sydney and the fact that his daughter Jude and family lived in southern New South

Wales, the decision not to move to Adelaide was a wise one, but Alan paid many visits to see his daughter Mandy, who was a Research Scientist in CSIRO Plant Industry at the Waite campus. Alan also kept up his friendship with Andrew and Sally Smith and they all shared several holidays, the last being on Kangaroo Island the year before Alan died, and when his health had severely declined.

Professional Activities

After his election to the Australian Academy of Science in 1982 Alan served the Academy in several different capacities, most notably as Ordinary Member of Council (1984–7) and Vice-President of Council (1986–7). Membership of other Academy committees included the National Committee on Research Funding (1984–5), the Plant Sciences Sectional Committee that short-lists candidates who fall in that category (1989–92), the School Science Committee (1987–93) and the Biology for the Nineties Project (1990–3). Alan was a member of the Advisory Committee for the *Australian Journal of Plant Physiology* (1984–9). Alan had a strong interest in science education at secondary school level and in the 1980s and 1990s was a member of several committees within the New South Wales science examination system, particularly for biology. He served in many capacities at the University of Sydney, including Head of School and on the Academic Board and Senate, where he became active in University politics. He was also heavily involved in the formation of the Australian Society for Biophysics and was its first President (1975–6).

External Pursuits

After Alan returned to Australia he developed an extraordinarily wide range of interests that spanned C. P. Snow's two cultures of the humanities and sciences. He pursued and successfully juggled these interests with great determination. Alan had a correspondingly wide range of close friends and there are many fond memories—far too many to cover here. All of us would agree with the comment by John Burnheim, a former Professor of Philosophy at the University of Sydney, and a close friend with whom Alan shared ownership of a small yacht for several years, that Alan was a very complex person.

John astutely doubted whether any of Alan's friends could respond adequately to every aspect of his rich personality. According to John: 'The core of his identity was remarkably consistent and constant. But he escaped conventional categories, the unexamined assumptions of everyday banality.' For example, Alan was politically very much on the left but he was by no means uncritical, and strongly disliked the bureaucratic and legal restrictions that were commonly the reaction to the monetary focus of capitalism. He was strongly opposed to Australia's involvement in the Vietnam War and in any invasion of other countries. Alan was a strong republican but admitted that he voted 'no' in the 1999 republic referendum, one reason being that he disapproved of the pro-republic case that the President should be elected by the parliament, not by the people. He also disapproved of the beginning of the proposed new Preamble to the Constitution ('With hope in God...').

Aesthetics

Following the family's return to Australia, Alan was for a long time a lover of Citroën cars, revelling in their elegance and the quirkiness of their controls—though the quirkiness was not always appreciated by others when called on to drive his car for one reason or another. Alan showed his elegance by his clothes which, along with his other possessions he chose (again quoting John Burnheim) 'so that he was completely in harmony with them. They expressed a deep unity between his appearance, his life-style and his personality.' His friend Anne Warren once commented that Alan could look elegant in jeans and a tee-shirt, though a fondness for brightly coloured socks and Alan's red-green colour blindness did sometimes temper the elegance below the trouser bottoms. Alan's choice of clothing did not include ownership of a dinner suit, and he also refused to hire one, so that on principle he never attended the 'black tie' annual dinner of the Australian Academy of Science.

Alan had very definite aesthetic tastes. Ceramics were a particular passion, and he made every effort to meet potters whose work he admired, who included the celebrated Dame Lucie Rie, Gwyn Hanssen Pigott, Col Levy, Milton Moon and Peter Rushforth. He built up a valuable collection over many years, developing a taste for the most modern ceramics

including Yasu Koyama's work. He was a dedicated photographer and spent many hours working out the physics of every lens. Alan was a keen observer of the visual arts including the French impressionists, Monet in particular. He frequented the Art Gallery of New South Wales and the White Rabbit Gallery in Sydney and enjoyed intellectual discussions on art. Asked once how his red-green colour blindness affected what he could see in paintings, the response was that he interpreted all the colours in his mind's eye. Alan's favourite author was Samuel Becket, though he read very widely and was intrigued by descriptions of food in novels. Although he had wide interests in music, from the baroque period to the Beatles, they certainly did not include anything romantic (whether composition or performance).

Alan was also—to use a word that he would have hated—a 'foodie', both in the sense of enjoyment in cooking carefully chosen meals, and when eating in restaurants. He was also very interested in wine and took great pleasure in influencing his friends in this regard, though many needed no encouragement. Alan was especially fond of Chinese food, and had favourite haunts in Sydney's Chinatown, to which he took visiting friends on the nights when he was not at work in the kitchen at home.

Bridging Cultures

Alan's tendency to switch focus between his interests could sometimes be alarming to his collaborators, and no doubt contributed to the view of some of his PhD students that he could be a distant supervisor even when at home in Sydney. Dale Sanders, another of Enid MacRobbie's former PhD students, who later moved to the University of York and thence to the John Innes Institute (and now FRS), recalls that after Alan's arrival in York for a 6-week research trip, he immediately departed for five weeks of touring the art galleries of Europe. However, Alan completed the scientific assignment in the final week with his pocket holder for *Chara* cells and his laptop computer. John Patrick recalls that meetings arranged to discuss research had a prelude of non-scientific matters, preferably over a cup of coffee. Topics included discourses on one or more of Alan's numerous passions that included the finer points of Japanese ceramics, the use of wood-fired ovens for glazing certain

clays, palaeontology digs in central Queensland and elsewhere, bird-watching and his beloved granddaughters. John's colleagues affectionately referred to 'Uncle Alan'.

Although Alan travelled widely, his first visit to Japan was not until 1993, when he went to a membrane transport conference in Kyoto with Andrew Smith, who had been to Japan previously, and who observed with interest an unusual uncertainty in Alan about this unfamiliar environment. Nevertheless, Alan was determined to sample Japanese food very thoroughly and for the first hotel breakfast the day before the conference started he ordered the Japanese breakfast. He was disconcerted when it arrived with the various items neatly set out on a tray because he did not know in which order they should be eaten. Andrew could not help—although he very much likes Japanese food in general, this does not extend to breakfast. Looking round at the others (all Japanese) in the restaurant for some guidance did not help at all—everyone was eating the Western breakfast: scrambled eggs, the lot. Nevertheless, Alan soon added local Japanese cuisine to his preferences (and cooking skills). Alan also found time to visit some of the temples and shrines in Nara and Kyoto and this inevitably led to a new enthusiasm for Japanese culture and to further visits that extended his appreciation of contemporary ceramics.

Alan in the Field

Alan was introduced to scientific field work when he first helped Anne Warren, who researched early vertebrate palaeontology at La Trobe University, on a fossil-finding trip in Queensland. According to Anne, this experience was preceded by 'a huge interest in finding the best camping gear, the most sun-proof shirts and hats, and later, camp cooking. He produced a camp cook book.' The experience led to a strong interest in palaeontology, despite frustration when his colour blindness sometimes made it difficult to distinguish fossils from the rocks in which they were embedded. Again according to Anne, he marked his hammer, chisel, and sitting mat with red/yellow/black tape in a tribute to the traditional owners of the fossil site.

When Anne was researching early vertebrate footprints found on a sandstone rock slab in the courtyard of a historic homestead in the Grampians, Victoria, Alan spent weeks in the



Figure 1. Alan Walker and Sally Smith camping at Bool Lagoon, South Australia, 1993. Photo: Andrew Smith.

Mitchell Library in Sydney trying to find the origin of the sandstone, which had been quarried soon after Major Mitchell explored the area in the 1830s. He found old maps and diaries that eventually led them to the quarry. He was still updating Anne Warren on the latest vertebrate fossils from the Early Carboniferous early in 2013.

It was apparently this field work that led Alan to a keen interest in four-wheel drive vehicles (Land Rover Discovery) and bird-watching, which he embraced passionately, and led to many excursions to birding sites in Australia. Some of these holidays were shared with Sally and Andrew Smith (Fig. 1). They recall that on the first one there was one pair of small binoculars

between them and later, when they all had binoculars, Alan was the first to buy a telescope. He invited other friends to share camping trips. Bill Allaway (formerly in the School Biological Sciences, University of Sydney) recalls that he and his wife Anne Ashford (formerly in the School of Biological, Earth and Environmental Sciences, University of New South Wales) took part in one of the inland camping trips, 'which became a truly memorable experience of birds, out-back soundscapes, aboriginal stone carvings and paintings, all shared with his composer friends David Lumsdaine and Nicola LeFanu and their son.' Aboriginal art was another particular interest of Alan's and he was greatly disappointed when his developing illness made him cancel a long-planned expedition to the Kimberley region in Western Australia. One of his favourite trips to reminisce about was to La Perouse Bay, Manitoba, Canada, on the shores of Hudson Bay, Manitoba in the mid-1990s. To get to and from the compound, a helicopter ride from Churchill was the preferred method. He was fascinated by the huge landscape and the insignificance of humans, who were required to carry flares and guns to practice drills in case of attacks by bears.

Declining Eyesight

Sadly, Alan's eyesight suffered from macular degeneration and gradually declined, curtailing his bird-watching—much to his frustration. However, using his knowledge of the physics of optics he became particularly interested in pinhole photography and conducted a series of experiments. Alan's ophthalmologist, Professor Mark Gillies (University of Sydney), persuaded Alan that a cataract operation would improve his sight, and this led to a partial improvement and the regaining of his driving licence. From 2010 until 2013, and blind in one eye, Alan was a member of the Board of a highly successful program run under the auspices of the Royal Australian and New Zealand College of Ophthalmologists, and called 'Fight Retinal Blindness!' The project is dedicated to finding the best ways to preserve sight without unwanted side effects. Mark Gillies, leader of this project, regarded Alan as 'an astute observer, and a terrific bloke who always wore socks that did not match'.

Conclusions

Alan Walker leaves a fine legacy in science that was recognized at a meeting of the Plant Transport Group, part of the UK Society of Experimental Biology, in December 2014. This group was established in the 1980s to provide a forum for research scientists early in their careers to present and discuss their work. It covers the molecular biology, biochemistry, biophysics and physiology of plant membranes and it is very significant that there was a session in which highlights of Alan's long career were described by Mary Beilby and Dale Sanders and which fascinated the delegates.

Alan was indeed a complex man and his many friends have quite different things by which to remember him. There is an important comment by John Burnheim that 'Alan was a genuine male feminist, encouraging his daughters and his students towards independence, including independence of him, and attracting a diverse group of friends among women'—both within and beyond science.

Alan's friends would surely agree with another comment by John: 'He could talk illuminatingly about many topics, but never came across as a know-all. He knew and accepted his own limitations, understanding that all important knowledge and art is very precise and specific, never vague and amorphous.' Interestingly, he regarded some of his research collaborators as intuitive as regards consideration of previous work when it came to deciding what to do next, something that they found surprising. This was by no means a criticism—it reflected Alan's own need for clear logical reasons that satisfied him before continuing. The results were carefully designed experiments and well described questions and outcomes in papers where he was an author.

Alan died on 27 October 2013. His marriage to his wife Rona was dissolved in 1975. As well as his brother Geof, Alan is survived by his beloved daughters Jude and Mandy, and his very precious grand-daughters Suzannah and Lucinda.

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to John Burnheim, who collated the moving tributes and reminiscences to Alan that were distributed at the celebration of his life. We have greatly depended on some of these, especially John's and those by Bill Allaway and Anne Warren, in order to give what we hope is a well-balanced picture of 'Alan, the man'. Bill Allaway, Mark Gillies, John Patrick, Dale Sanders and Sally Smith also gave us useful material and we thank Sally for her comments on the draft. The portrait of Alan in retirement was taken by Emeritus Professor Michael Gal (School of Physics, University of New South Wales) in 2008. Writing this memoir has been quite an emotional experience, and Andrew Smith apologizes for the delay, for which he was responsible.

Endnotes

- ¹ N. A. Walker, 'Microelectrode Experiments on *Nitella*', *Australian Journal of Biological Sciences*, 8 (1955), 477–489.
- ² N. A. Walker, 'Ion Permeability of the Plasmalemma of the Plant Cell', *Nature*, 180 (1957), 94–95.
- ³ N. A. Walker, 'Ion Permeability of the Plasmalemma of the Plant Cell', *Nature*, 181 (1958), 1288–1289.
- ⁴ A. B. Hope and N. A. Walker, 'Ionic Relations of Cells of *Chara australis*, IV, Membrane Potential Differences and Resistances', *Australian Journal of Biological Sciences*, 14 (1961), 26–44.
- ⁵ N. A. Walker and A. B. Hope, 'Membrane Fluxes and Electric Conductance in Characean Cells', *Australian Journal of Biological Sciences*, 22 (1969), 1179–1195.
- ⁶ G. P. Findlay, A. B. Hope, M. G. Pitman, F. A. Smith and N. A. Walker, 'Ionic Fluxes in Cells of *Chara corallina*', *Biochimica et Biophysica Acta*, 183 (1969), 565–576. G. P. Findlay, A. B. Hope, M. G. Pitman, F. A. Smith and N. A. Walker, 'Ionic Relations of Marine Algae, III, *Chaetomorpha*', *Australian Journal of Biological Sciences*, 24 (1971), 731–745. G. P. Findlay, A. B. Hope, M. G. Pitman, F. A. Smith and N. A. Walker, 'Ionic Relations of the Marine Alga *Valoniopsis pachynema*', *Australian Journal of Plant Physiology*, 5 (1987), 675–686.
- ⁷ A. B. Hope and N. A. Walker, *The Physiology of Giant Algal Cells* (Cambridge University Press, Cambridge, 1975).
- ⁸ N. A. Walker and F. A. Smith, 'Intracellular pH in *Chara corallina* Measured by DMO Distribution', *Plant Science Letters*, 4 (1975), 125–132.
- ⁹ F. A. Smith and N. A. Walker, 'Chloride Transport in *Chara corallina* and the Electrochemical Potential Difference for Hydrogen Ions', *Journal of Experimental Botany*, 27 (1976), 451–459. N. A. Walker and F. A. Smith, 'The H⁺ ATPase of the *Chara* Cell Membrane: its Role in Determining Membrane PD and Cytoplasmic pH', in *Transmembrane Ionic Exchanges in Plants*, eds. M. Demarty *et al.* (Université de Rouen, 1977), pp. 254–264.
- ¹⁰ N. A. Walker, M. J. Beilby and F. A. Smith, 'Amine Uniport at the Plasmalemma of Charophyte Cells: I. Current-voltage Curves, Saturation Kinetics, and Effects of Unstirred Layers', *Journal of Membrane Biology*, 49 (1979), 21–55. N. A. Walker, F. A. Smith and M. J. Beilby, 'Amine Uniport at the Plasmalemma of Charophyte Cells: II. Ratio of Matter to Charge Transported and Permeability of Free Base', *Journal of Membrane Biology*, 49 (1979), 283–296.
- ¹¹ F. A. Smith and N. A. Walker, 'Photosynthesis by Aquatic Plants: Effects of Unstirred Layers in Relation to Assimilation of CO₂ and HCO₃⁻ and to Carbon Isotopic Discrimination', *New Phytologist*, 86 (1980), 245–259.
- ¹² F. A. Smith and N. A. Walker, 'Chloride Transport in *Chara corallina* and the Electrochemical Potential Difference for Hydrogen Ions', *Journal of Experimental Botany*, 27 (1976), 451–459.
- ¹³ M. J. Beilby and N. A. Walker, 'Chloride Transport in *Chara*: I. Kinetics and Current-voltage Curves for a Probable Proton Symport', *Journal of Experimental Botany*, (1981), 53–54.
- ¹⁴ N. A. Walker and F. A. Smith, 'Circulating Electric Currents between Acid and Alkaline Zones Associated with HCO₃⁻ Assimilation in *Chara*', *Journal of Experimental Botany*, 28 (1977), 1190–1206. N. A. Walker, F. A. Smith and I. R. Cathers, 'Bicarbonate Assimilation by Fresh-water Charophytes and Higher Plants: I. Membrane Transport of

- Bicarbonate Ions Is Not Proven', *Journal of Membrane Biology*, 57 (1980), 51–58.
- ¹⁵ M. A. Bisson and N. A. Walker, 'The *Chara* Plasmalemma at High pH: Electrical Measurements Show Rapid Specific Passive Uniport of H⁺ or OH⁻', *Journal of Membrane Biology*, 56 (1980), 1–7. M. A. Bisson and N. A. Walker, 'Control of Passive Permeability in the *Chara* plasmalemma', *Journal of Experimental Botany*, (1982), 520–532.
- ¹⁶ T. E. Bostrom and N. A. Walker, 'Intercellular Transport in Plants. I. The Rate of Transport of Chloride and the Electric Resistance', *Journal of Experimental Botany*, 26 (1975), 767–782. T. E. Bostrom and N. A. Walker, 'Intercellular Transport in Plants. II. Cyclois and the Rate of Intercellular Transport in *Chara*', *Journal of Experimental Botany*, 27 (1976), 347–357.
- ¹⁷ P. T. Smith and N. A. Walker, 'Studies on the Perfused Plasmalemma of *Chara corallina*. I. Current-voltage Curves: ATP and Potassium Dependence', *Journal of Membrane Biology*, 60 (1981), 223–236.
- ¹⁸ S. E. Smith and N. A. Walker, 'A Quantitative Study of Mycorrhizal Infection in *Trifolium*: Separate Determination of the Rates of Infection and of Mycelial Growth', *New Phytologist*, 89 (1981), 225–240.
- ¹⁹ R. J. Reid and N. A. Walker, 'Adenylate Concentrations in *Chara*: Variability, Effects of Inhibitors and Relationship to Protoplasmic Streaming', *Australian Journal of Plant Physiology*, 10 (1983), 373–383.
- ²⁰ R. J. Reid and N. A. Walker, 'The Energetics of Cl⁻ Active Transport in *Chara*', *Journal of Membrane Biology*, 78 (1984), 35–41. R. J. Reid and N. A. Walker, 'Control of Cl Influx in *Chara* by Inter al pH', *Journal of Membrane Biology*, 78 (1984), 157–162.
- ²¹ J. R. Smith and N. A. Walker, 'Membrane Conductance of *Chara* in the Acid and Basic Zones', *Journal of Membrane Biology*, 78, 232–233. J. R. Smith and N. A. Walker, 'Effects of pH and Light on the Membrane Conductance Measured in the Acid and Basic Zones of *Chara*', *Journal of Membrane Biology*, 83 (1985), 193–205.
- ²² J. R. Smith, F. A. Smith and N. A. Walker, 'Potassium Transport Across the Membranes of *Chara*: I. The Relationship between Radioactive Tracer Influx and Electrical Conductance', *Journal of Experimental Botany*, 38 (1987), 731–751. J. R. Smith, N. A. Walker and F. A. Smith, 'Potassium Transport Across the Membranes of *Chara*: III. Effects of pH, Inhibitors and Illumination', *Journal of Experimental Botany*, 38 (1987), 778–787.
- ²³ K. A. Fairley and N. A. Walker, 'Amine Ion Porter in *Chara australis*: Effects of Alkyl Substitution and External pH', *Journal of Membrane Biology*, 98 (1987), 191–196.
- ²⁴ P. R. Ryan and N. A. Walker, 'Malate Accumulates in the Vacuole of *Chara australis* during Uptake of Ammonium from Chloride-free Solution', *Journal of Experimental Botany*, 44 (1993), 637–643. P. R. Ryan and N. A. Walker, 'The Regulation of Ammonia uptake in *Chara australis*', *Journal of Experimental Botany*, 45 (1994), 1057–1067.
- ²⁵ M. R. Wilson and N. A. Walker, 'The Transport and Metabolism of Urea and its Analogues in *Chara australis*. I. Passive Diffusion and Specific Transport', *Journal of Experimental Botany*, 39 (1988), 739–752. M. R. Wilson, S. I. O'Donohue and N. A. Walker, 'The Transport and Metabolism of Urea and its Analogues in *Chara australis*. III. A Cotransport System and a Second Specific System for Urea Transport', *Journal of Experimental Botany*, 39 (1988), 763–774.
- ²⁶ N. A. Walker, R. J. Reid and F. A. Smith, 'The Transport and Metabolism of Urea and its Analogues in *Chara australis*. IV. Sodium Symport—a Slip Model for the High and Low Affinity Systems', *Journal of Membrane Biology*, 136 (1993), 263–271.
- ²⁷ H. Lühring, 'Recording of Single K⁺ Channels in the Membrane of Cytoplasmic Drop of *Chara australis*', *Protoplasma*, 133 (1986), 19–27.
- ²⁸ D. R. Laver, C. A. Cherry and N. A. Walker, 'The Actions of Calmodulin Antagonists W-7 and TFP and of Calcium on the Gating Kinetics of the Calcium-Activated Large Conductance Potassium Channel of the *Chara* Protoplasmic Drop: a Substate-sensitive Analysis', *Journal of Membrane Biology*, 155 (1997), 263–274.
- ²⁹ F. A. Smith and N. A. Walker, 'Transport of Potassium in *Chara australis*, I. A Symport

- with Sodium', *Journal of Membrane Biology*, 108 (1989), 125–137.
- ³⁰ S. R. McCulloch, M. J. Beilby and N. A. Walker, 'Transport of Potassium in *Chara australis*: II. Kinetics of a Symport with Sodium', *Journal of Membrane Biology*, 115 (1990), 129–143.
- ³¹ F. J. M. Maathuis, D. Verlin, F. A. Smith, D. Sanders, J. A. Fernandez and N. A. Walker, 'The Physiological Relevance of Na⁺-coupled K⁺-transport', *Plant Physiology*, 112 (1996), 1609–1616.
- ³² S. E. Smith, S. Dickson and N. A. Walker, 'Distribution of Mycorrhizal entry-points near the Root Apex: Is there an Uninfectible Zone near the Root tip of Leek and Clover Roots?', *New Phytologist*, 122 (1992), 469–477.
- ³³ T. L. Holdaway-Clarke, N. A. Walker, P. K. Hepler and R. L. Overall, 'Physiological Elevations in Cytoplasmic Free Calcium by Cold or Ion Injection Result in Transient Closure of Higher Plant Plasmodesmata', *Planta* 210 (2000), 329–335.
- ³⁴ M. J. Beilby and N. A. Walker, 'Modeling the Current-voltage Characteristics of *Chara* Membranes. I. The Effect of ATP Removal and Zero Turgor', *Journal of Membrane Biology*, 149 (1996), 89–101.
- ³⁵ S. Al Khazaaly, N. A. Walker, V. A. Shepherd and M. J. Beilby, 'Membrane Potential Fluctuations in *Chara australis*: a Characteristic Signature of High External Sodium', *European Biophysics Journal*, 39 (2009), 167–174.
- ³⁶ Mary J. Beilby and Michelle T. Casanova, *The Physiology of Characean Cells*, (Springer: Berlin, 2014).
- ³⁷ N. A. Walker, J. W. Patrick, W-H. Zhang and S. Fieuw, 'Efflux of Photosynthate and Acid from Developing Seedcoats of *Phaseolus vulgaris* L.: a Chemiosmotic Analysis of Pump-driven Efflux', *Journal of Experimental Botany*, 46 (1995), 539–549.
- ³⁸ W-H. Zhang, B. J. Atwell, J. W. Patrick and N. A. Walker, 'Turgor-dependent Efflux of Assimilates from Coats of Developing Seed of *Phaseolus vulgaris* L.: Water Relations of the Cells Involved in Efflux', *Planta*, 199 (1996), 25–33.
- ³⁹ J. W. Patrick, W-H. Zhang, S. D. Tyerman, C. E. Offler and N. A. Walker, 'Role of Membrane Transport in Phloem Translocation of Assimilates and Water', *Australian Journal of Plant Physiology*, 28 (2001), 695–709.
- W-H. Zhang, N. A. Walker, J. W. Patrick and S. D. Tyerman, 'Calcium-dependent K Current in Plasma Membranes of Dermal Cells of Developing Bean Cotyledons', *Plant, Cell and Environment*, 27 (2004), 251–262.
- ⁴⁰ N. A. Walker, H. A. L. Henry, D. J. Wilson and R. L. Jefferies, 'The Dynamics of Nitrogen Movement in an Arctic Salt Marsh in Response to Goose Herbivory: a Parameterized Model with Alternate Stable States', *Journal of Ecology*, 91 (2003), 637–650.
- ⁴¹ R. L. Jefferies, N. A. Walker, K. A. Edwards and J. Dainty, 'Is the Decline of Soil Microbial Biomass in Late Winter Coupled to Changes in the Physical State of Cold Soils?', *Soil Biology and Biochemistry*, 42 (2010), 129–135.