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TRIBUTE

Reminiscences of work with Alex Hope: the movement of water and ions in giant algal cells, 1963–1967

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Abstract This article, from the Tribute to Alex Hope Symposium at the 2008 Australian Society for Biophysics meeting, represents reminiscences of PhD studies done under my former supervisor, Professor Alex Hope. The studies demonstrated and quantified electroosmosis in giant algal cells of Chara and isolated segments of cell wall by measuring instantaneous current-induced volume flows. The studies also uncovered and modelled an unstirred-layer transport number effect that gave rise to an additional transiently increasing current-induced volume flow component, which could be mistaken for electroosmosis. In addition, action potential induced volume flows and pressure changes were measured in these cells and successfully modelled. An appreciation of the influence of Alex Hope and his laboratory environment, together with some of the further studies that resulted from this work, is also mentioned.

Keywords Chara cells · Electroosmosis · Unstirred layers · Transport numbers · Action potential induced volume flows · Action potential induced pressure changes

"Proteins, membranes and cells: the structure-function nexus". Contribution from a special symposium in honour of Professor Alex Hope of Flinders University, South Australia held during the annual scientific meeting of the Australian Society for Biophysics, Canberra, ACT, Australia, September 28–October 1, 2008.

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When I commenced my PhD in 1963, under the supervision of Professor Alex Hope in the Joint Plant Physiology Unit (PPU) in the Botany School of the University of Sydney, the other occupants of the lab were Dr Geoff Findlay and an MSc/PhD student, Hans Coster (see also Barry et al. 2009). My first impressions of Alex were of a friendly but reserved person of integrity, who wanted to make sure that I was prepared to take on all that was involved in a PhD project and a potential research career, before he agreed to take me on as a student. At that time, Alex and Geoff were completing a series of highly cited papers on the action potential in the giant algal cell, *Chara australis* (e.g., Hope 1961; Findlay and Hope 1964), and Alex had recently completed an excellent research monograph (Briggs et al. 1961). This monograph was a most helpful introduction, particularly for a physicist, to plant cells, electrophysiology and biophysics. It was presented with mathematical rigour from a physico-chemical perspective, with membrane potential equations derived from electrochemical potentials, and it included topics such as diffusion equations for electrolytes, surface charge effects, electrical double layers and unstirred-layer effects.

My initial project was to see if electroosmotic flows could be measured across the membranes of the above *Chara* cells, to determine whether ions moved through aqueous channels and were able to drag water molecules with them. These single *Chara* cells, about 8–15 cm long with a diameter of about 1 mm, had an internal osmotic pressure of 8–10 atmospheres in dilute electrolyte solutions and behaved like rigid cylinders. In the lab, each of our setups included a Faraday cage, microscope, Keithley electrometer and an industrial chart recorder, which we had to modify ourselves, with a clutch drive to give us a fast speed. Other items we had to make had the backup of a good mechanical workshop, if it was required. For

example, electronic circuits, such as the amplifier for the phototransducer system, I needed to develop for measuring minute volume flows, which used fairly newly developed transistors, required getting a circuit design from an electrical engineer, whom Alex knew, and then building it. The phototransducer system was able to continuously measure nanolitre transcellular flows through either a whole algal cell or a segment of cell wall by means of a bead of Hg, resting within a column of dilute saline in a clean glass capillary tube, partially obstructing a light beam falling on a solar cell (Fig. 1), with its output connected to the amplifier described above (see Barry and Hope 1969b). This equipment enabled the measurement of reasonably large transcellular volume flows resulting from an osmotic gradient and also extremely small volume flows seen to accompany current flows.

Of general interest, I recall one project of Alex with Hans and a research assistant in the lab later on during my PhD, which illustrates Alex's innovation and interest in new technology. This was the building of a very early analogue computer to solve radioactive tracer differential equations (see details in Barry et al. 2009).

In terms of my memories of Alex as a supervisor, he left me to get on with my research on my own, while he busily worked in his office down the corridor or, earlier on, did some tracer flux experiments in the lab. In those days in Sydney, he gave the impression of being a little distant from PhD students, with little direct interaction between him and PhD students like Hans and me, on either research or other matters. However, Alex was very helpful in providing me with critical reprints and suggested references, especially at the early stages of the project, and in checking periodically how the project was going. And I do remember musing, when he was on study leave in the UK in 1963–1964, that I had more research interaction with him by correspondence, with some long airmail letters, than I had when he was present in the lab, though I never considered any lack of direct interaction with Alex to be a problem. It should be added that when we later moved to Flinders University, he seemed much less distant, and I remember him helping me weld some of our Faraday cages and tables together, to get experiments up and running as quickly as possible.

Returning to my PhD experiments, I found that the transcellular osmotic volume flow through a Chara cell, resulting from a complete bulk replacement of a 0.1 mM KCl solution in the open chamber to 1.0 mM KCl, was initially maximal and then it decayed transiently. The volume flow then reversed when the external solution was replaced by 0.1 mM KCl, before returning almost back to its original volume (cp. Fig. 16 of Barry and Hope 1969b). Similar shaped time courses and reversed flows had been previously obtained with a very large 0.2 M transcellular sucrose gradient by Tazawa and Nishizaki (1956; cp. their Fig. 2) using manual capillary measurements. In contrast, I eventually found that when currents were passed across the cell membranes, that there was a very small resultant volume flow, but that it had a different time-dependence. The volume flow rate continued to increase for a few minutes during the passage of current (see Fig. 13 of Barry and Hope 1969b). In 1965, around the time of these experiments, Alex Hope and Mike Pitman, with their well-established international connections, had organised an excellent international conference on "The Physiology of Giant Algal Cells" in the Australian Academy of Science Dome building in Canberra, which included leading international plant and animal biophysicists of that period, such as Richard H. Adrian, Richard D. Keynes, Enid A. C. McRobbie, Jack Dainty, N. Alan Walker, Lorin J. Mullins, Uichiro Kishimoto, RN (Bob) Robertson and Geoff Findlay, together with PhD students, Hans Coster and myself. When my current-induced transient volume flow results were shown in a talk at the conference, Richard Adrian suggested that the transient response might be due to transport number effects in a three-compartment system,

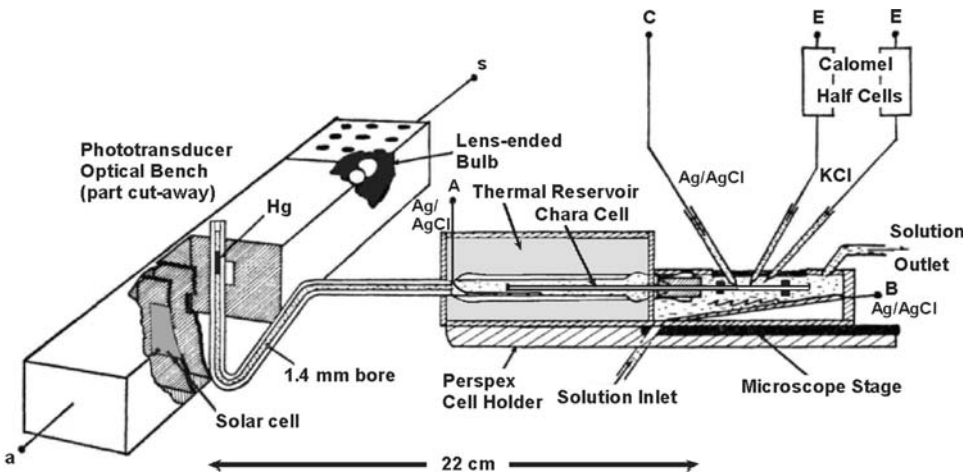

Fig. 1 A schematic diagram of the final cell holder and phototransducer system used for measuring current-induced volume flows in whole *Chara* cells and cell walls. Modified from Fig. 2 of Barry and Hope (1969b), where further experimental details are given

where the transport number of an ion is the fraction of current carried by that ion in a particular phase (e.g., membrane or solution). Being well aware of the work of Jack Dainty on unstirred layers (USLs) adjacent to plant cell membranes, it became clear to me that differences in transport numbers for ions between membranes and adjacent solutions in the presence of USLs should give rise to time-dependent local concentration changes within the solution at any membrane–solution interface and that the principle should also apply to a simple planar membrane, such as a segment of highly negatively charged cation-selective cellulose plant cell wall. True electroosmosis should result in an instantaneous current-induced volume flow (Barry and Hope 1969b), whereas USL transport number effects would give rise to a time-dependent component. The aim was therefore (1) to see whether such an instantaneous component could be discerned by improving the time resolution of the recording system and (2) whether the USL component could be calculated and perhaps even indirectly confirmed. Figure 2 shows an example of a typical result in a whole plant cell, where a clear instantaneous change in volume flow component occurred within a fraction of a second after the onset, and after the termination, of the current. Figure 3 shows a special stopper used to hold a segment of plant cell wall and Fig. 4 shows fast recordings across a planar cell wall segment. Again, the initial change in slope at the onset and termination of the current pulse in the fast recording indicates that there is a true electroosmotic component present, whereas the transient changes suggest an additional USL component.

A significant part of this project involved analysing the role of transport number effects and USLs in contributing to current-induced volume flows across the membranes of whole plant cells and across plant cell walls (Barry and Hope 1969a). The principles of the effect are illustrated in Fig. 5. As the wall (or cell membrane) will be predominantly permeable to K^+ ions, its transport number, t_K, will be ~ 1.0 and that of Cl^-, t_{Cl}, will be ~ 0 there, as indicated. However, in the adjacent KCl solution, approximately half

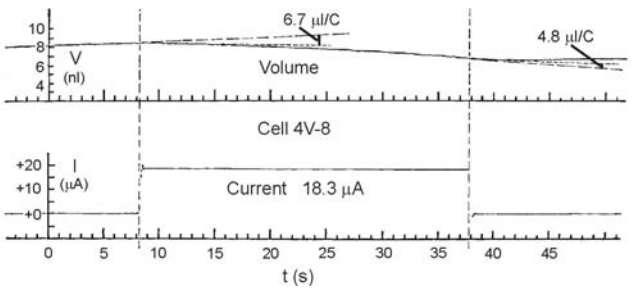

Fig. 2 A fast recording of volume flows, *V*, in nL showing typical initial and final jumps in flow rates (with electroosmotic coupling coefficients) through a cell of *Chara australis* in 1.0 mM KCl. Modified from Fig. 12 of Barry and Hope (1969b)

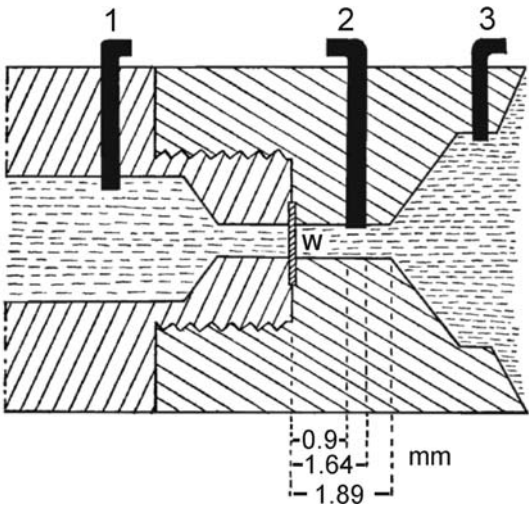

Fig. 3 A special double perspex (lucite) stopper used to hold a segment of plant cell wall (w), together with 3 Ag/AgCl electrodes for monitoring membrane potential and local Cl^- activity, a_{Cl}. Modified from Fig. 3 of Barry and Hope (1969b)

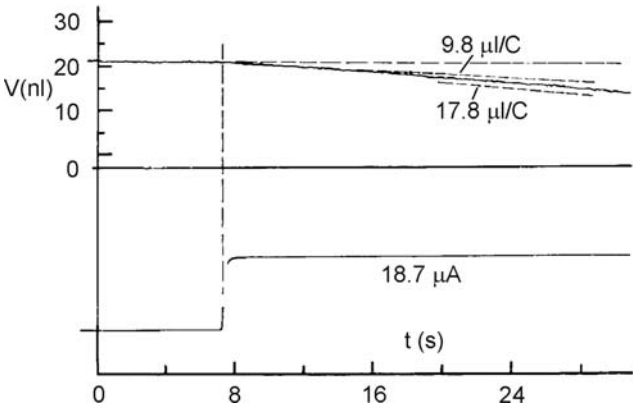

Fig. 4 Fast recordings of the volume, *V* (nl), and the current I (µA) for volume flows across a planar segment of cell wall (W5 M-26), with initial coupling coefficient of 9.8 µl C^{-1}. Modified from Fig. 5b of Barry and Hope (1969b), where examples of slow recordings and further experimental details are given

of the current [$u_K/(u_K + u_{Cl})$, where u is the relative mobility of the ions] will be carried by each ion, $t_K \approx t_{Cl} \approx 0.5$. This means that for every two units of elementary charge crossing the membrane, one K^+ and one Cl^- ion will be added to the solution at the right wall–solution interface and one of each ion species will be removed from the solution at the left solution–wall interface. This will build up local salt concentration gradients as shown in Fig. 5 within the unstirred-regions of the stopper channels, which will be opposed by diffusion down each concentration gradient. The local osmotic concentration gradients, which will take a while to develop, will give rise to an osmotic volume flow, J_v (predominantly H_2O), in the same direction as the current. The local concentration gradients will also be reduced by any back diffusion of salt

across the wall and by sweeping-away effects of the volume flow, though the latter effect is likely to be quite small in the light of the salt concentrations present. The resulting equations were derived from first principles along the lines used by Carslaw and Jaeger (1959) for somewhat analogous heat transfer problems. An example of the sort of equations resulting from such an analysis for a segment of cell wall is given below, where the volume flow, J_v, due to both a true electroosmotic component, j_o, and a transient USL component is given (Barry and Hope 1969a; Eqs. 10 and 11) by:

$$J_v = j_o + \frac{4\sigma L_p RT\Psi}{D}\left\{\frac{\ell}{1+\beta\ell} - 2\sum_m \frac{e^{-D\alpha_m^2 t}}{[(\ell\alpha_m^2+\beta^2)+\beta]}\right\} \quad (1)$$

where the α_m are the solutions of

$$\alpha_m \ell \cot \alpha_m \ell = -\beta\ell \quad (2)$$

σ, L_p and P_{KCl} are the reflection coefficient, hydraulic conductivity and KCl permeability of the cell wall; R,T and D are the gas constant, temperature in K and diffusion coefficient of KCl in solution; ℓ is the USL length, β is the solute back-diffusion parameter $= 2\ P_{KCl}/D$ and Ψ, the rate of KCl generation at each wall-solution interface, is $= \alpha I/F$, where I and F are the current density and Faraday, α is the change in transport number between the cell wall (w) and solution (s) [i.e., $\alpha = t_K^w - t_K^s = -(t_{Cl}^w - t_{Cl}^s) \approx 0.5$] and t is the time. The solutions for the cylindrical cell membranes resulted in somewhat similar structured expressions with Bessel functions equations instead of trigonometric ones (Barry and Hope 1969a).

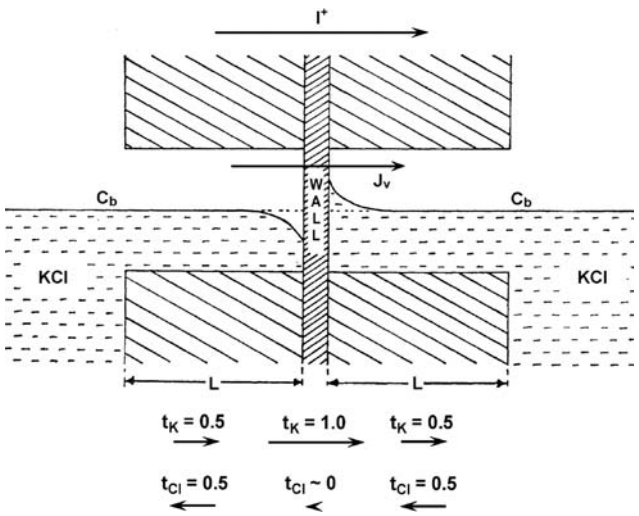

Fig. 5 The transport number effect—illustrating how a current can give rise to local concentration changes in USLs formed in the special stopper used to hold segments of cell walls shown in Fig. 3. The regions within the stopper channels will be considered to be unstirred and those beyond perfectly stirred. Modified from Fig. 3 of Barry and Hope (1969a)

As indicated in Barry and Hope (1969a, b), the theoretical predictions for the transient current-induced volume flow across both cell walls and membranes of whole cells agreed well with the experimental measurements. In addition, measurements of changes in Cl^- concentrations near cell walls, with Ag/AgCl electrode 2 (Fig. 3), gave values similar to those predicted theoretically. Furthermore, the USL nature of the effect was demonstrated directly by flushing the solution near the membrane with bulk solution during a current pulse and demonstrating an immediate drop in the volume flow (Fig. 11 in Barry and Hope 1969b). The overall conclusions of these papers were (1) that there was an electroosmotic coupling with coefficients of $\sim$38 H_2O molecules/ion for the intact cell membranes and of $\sim$53 for the cell walls and (2) that the USL components were $\sim$62 H_2O molecules/ion for the cell membranes and $\sim$59 for the cell walls, in each case being due to transport number effects.

A further analysis, with a major input from Alex Hope, of the contribution of the cell wall to the electroosmotic component of the intact cell membrane (combined cell membrane and attached cell wall) indicated that the cell wall would only have a minor contribution, reducing the coefficient from 38 to 31 H_2O molecules/ion for the membrane by itself in the absence of a cell wall (Barry and Hope 1969c).

During my PhD studies, two other experiments were also done. With the setup in Fig. 1, I was able to measure volume flow transients accompanying an action potential (an example is shown in Fig. 6a). On average, this represented an outflow of about 0.9 ± 0.1 nl s^{-1} cm^{-2} (total cell area $\sim$1 cm^2), with its peak lagging behind the action potential peak by about 0.2 s (Barry 1970a). It also seemed reasonable that such volume flows should produce a pressure change in the cells and that the deflection of a thin weighted wedge resting transversely across a cell might be sensitive enough to measure such changes. A theoretical analysis of the expected deformation of such a wedge given typical elastic moduli of the cell wall indicated the following simple relationship between wedge deflection and turgor pressure (P) of the cell (Barry 1967, 1970a).

$$P = \pi - \pi_o = \alpha\frac{Mg}{aX}, \quad (3)$$

where π and π_o are the osmotic pressures of the external solution and cell sap, respectively, M is the mass added to the wedge arm (Fig. 7), g is the gravitational constant, a is the cell radius, X is the deflection of the wedge, and α is a dimensionless constant calculated to be about 0.4. This relationship was verified experimentally for a cell in different osmotic solutions. From Eq. 3, for a small change

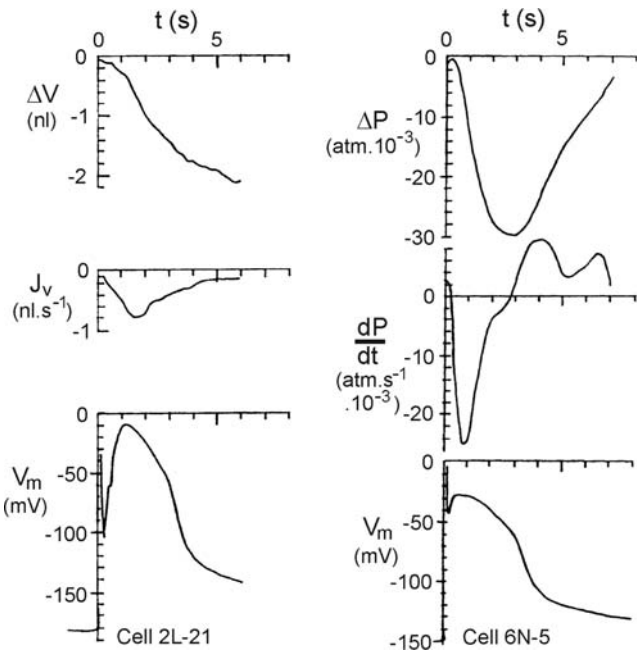

Fig. 6 Examples of a typical change in volume (ΔV) and volume flow J_v (=dV/dt) (**a**) and change in turgor pressure (P) and dP/dt (**b**) accompanying an action potential (V_m) in two *Chara* cells. Modified from parts of Figs. 6 and 10 of Barry (1970a)

in pressure, $\Delta P/P = -\Delta X/X$. An example of the measured pressure changes accompanying an action potential in *Chara* cells is shown in Fig. 6b, with the peak change in pressure (ΔP) being about 20×10^{-3} atm (equivalent to about 15 mmHg) with the peak value of dP/dt leading the action potential peak by about 0.1 s (Barry 1970a).

A theoretical analysis of action potential induced volume flows indicated that they could be explained by an increase in cell membrane P_{KCl} and a resulting efflux of KCl from the cell (Barry 1970b). This KCl efflux would then mainly produce (1) a volume outflow from the cell due to the frictional coupling of water with the flux of the two ions, estimated from electroosmotic coupling to be about 0.6 nl s^{-1} cm^{-2}, and (2) a local increase in the KCl concentration in the external USL with a resultant local osmotic volume flow, estimated to be between about 0.3 and 1.0 nl s^{-1} cm^{-2} (Barry 1970b). In addition, the measured pressure changes during the action potential could be correctly predicted in magnitude and time course as the result of the above volume outflow from a cell which behaves as a hydraulically leaky elastic cylinder, with known wall elastic modulus, and thereby drops in pressure as a result (Barry 1970b). Alex was also a very humble person and a very generous supervisor, willing to stand back from the action potential papers and let me publish them as a sole author.

Fig. 7 A simplified schematic diagram of the pressure transducer setup. *CW* is a counterweight to balance the different torques of the two arms of the lever system (arm ratio ~4.2:1), *W* is the added weight, *X* is the deflection of the surface of the cell below the edge of a thin steel wedge at the short end of the lever arm and the optical bench is the same as that used for the volume flow measurements. Based on Fig. 2 of Barry (1970a)

While I would have liked to have done some more experiments, I recall Alex very wisely insisting that I stop experiments and write up what I had. To emphasise the point, he gave my experimental rig to a visiting Russian scientist, Dr Lev Vorobiev, for him to use—so my experiments really did stop immediately.

Some of the further studies resulting from the work on electroosmosis and USL effects in Alex's lab and published in the Biophysical Journal (Barry and Hope 1969a, b), which have so far been cited by over 200 papers, were: (1) a Welcome Trust Fellowship to work with Richard Adrian, whom I had met at Alex's 1965 Canberra Conference, in the Physiological laboratory of the University of Cambridge, which ended up applying the principles elucidated in those papers to solve an electrophysiological problem in skeletal muscle fibres (e.g., Barry and Adrian 1973); (2) an invitation to a Benzon Symposium in Copenhagen to talk about unstirred layer effects and volume flows across biological membranes (Barry 1981), and (3) an invitation with Jared Diamond to write a review on unstirred layers for Physiological Reviews (Barry and Diamond 1984).

I would finally like to pay a special tribute to Professor Alex Hope, as my PhD supervisor, for suggesting the basic topic, for his advice, support and encouragement during the project, for allowing me the freedom to persevere with difficult experimental techniques and very lengthy theoretical analyses, for the stimulating environment of his research group, with Hans Coster, especially, and Geoff Findlay, and for the international collaborations, including my postdoctoral position with Jared Diamond, which Alex, with his international reputation, had developed.

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