Series

What history tells us
XI.
The complex history of the chemiosmotic theory

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1. Introduction

As a student, I was fascinated – probably like many others – by the paradigm shift introduced by Peter Mitchell in the field of bioenergetics, and by the long fight –17 years, from 1961 to 1978 – that ensued before the new concept was accepted by the community of biologists. The goal was to elucidate the nexus between the formation of ATP and the source of the energy required to do so – either light (in the case of photosynthesis) or oxidation (in the case of the mitochondrial respiratory chain). Instead of looking for a direct link, Peter Mitchell postulated an indirect one. He hypothesised that an electrochemical potential generated by the asymmetric distribution of protons between the two sides of a membrane drove the formation of ATP from ADP and phosphate.

The fascination of the Mitchell hypothesis does not fade when one reads the abundant literature which has already been produced on this story (Gilbert and Mulkay 1984; Robinson 1997; Prebble and Weber 2003; Weber 2005). Indeed, the opposite is true. This extraordinary scientific adventure can be considered from many different points of view. The first focuses on the man, and his apparent solitary fight against the majority of specialists in the field. For personal and health reasons, Peter Mitchell abandoned an academic position at the University of Edinburgh to create a private research institute, Glynn in Cornwall. He was able to give this small, isolated research laboratory an excellent scientific productivity and a high visibility and to establish close links with the international scientific community. This success attracted attention. The unusual place given by Mitchell to theory in biology (an attitude that generated criticism among many eminent biochemists, including Hans Krebs) finds a more favourable echo among biologists today. In addition, the chemiosmotic hypothesis appears as a supra-molecular vision of cell energetics, opposed to the more reductionist approach of most biochemists. This too is something to which we are more sympathetic today. Philosophers of science have found seductive characteristics in the early interest of Peter Mitchell for philosophy and the philosophical questions of life which nurtured his later scientific writings. In addition, he suggested that the way he introduced his bold hypothesis – the chemiosmotic model –, and how this hypothesis resisted the numerous experiments designed to challenge it, was a wonderful example of the way science progresses according to Karl Popper. Finally, the strong claims made by R J P Williams for the paternity of the model, and the apparently unfair attitude of Mitchell towards him, added a zest of scandal, blurring the boundary between "the hero and the villain" (Williams 1999).

Have the abundant books and articles written by the actors or external observers answered all the questions and provided a clear-cut description of the events? My guess is that the answer is no, at least in part. This is for two different reasons. The first reason is obvious to anyone who attempts to read the different contributions: the field is difficult and stands at the boundary between physical chemistry, biochemistry and cell biology. More than that, the style and vocabulary favoured by Mitchell are unusual and make the reading of his articles quite challenging. I have the feeling that many commentators, including Leslie Orgel (Orgel 1999), did not read the original articles and simply trusted the conclusions reached by Mitchell, or the later general descriptions he made of the construction of his theory. The second reason is that between the precise description of the experiments and the 'global' description of models something is missing. That something is linked to the general state of knowledge at the time of the controversy.

Keywords. Chemiosmotic hypothesis; interdisciplinarity; Karl Popper; Peter Mitchell; theory construction

It has to do with an appreciation of what is acceptable as an explanation at a given time in a given field.

2. The chronology of events

Mitchell studied as a biochemist in Cambridge and this training had a major influence on his future work. He endorsed the biochemical vision of Sir Frederick Gowland Hopkins, then Head of the Department of Biochemistry at the University of Cambridge, on the organised and dynamic behaviour of molecules within cells. He established close ties with David Keilin, whose influence remained strong on his later work (Mitchell 1979; Prebble and Weber 2003). In the course of his study of the transport of small molecules within bacteria (with Jennifer Moyle, who remained his main collaborator at Glynn), he elaborated the concept of *vectorial* metabolism. Mitchell thought that there was a strong link between metabolism and the transport of metabolites across membranes. The presence of an enzyme in a membrane can modify and orient metabolic reactions: the ligand is bound on one side of the membrane and the product of the reaction is released on the other (Mitchell 1957).

The principle of vectorial chemistry was applied by Mitchell to explain oxidative and photo-phosphorylation in a famous *Nature* paper (Mitchell 1961). The link between the respiratory or photosynthetic chain and the production of ATP was not an energy-rich intermediate, actively but unsuccessfully sought by most biochemists since the mid-1950s (Slater 1953; more recently the sought-after intermediate has been termed the "twentieth century phlogiston"; Allchin 1997). Rather, it was the result of the vectorial organisation of the respiratory chain and the enzyme that catalysed ATP synthesis, ATPase. Localised in the membrane, the activity of ATPase was directed towards the *production* (as opposed to breakdown) of ATP by the unequal distribution on the two sides of the membrane of the H^+ and OH^- ions generated by the redox loops of the respiratory or photosynthetic chain. The energy provided by this asymmetric distribution of ions could be estimated by the electrochemical potential, the sum of the chemical and electrical potentials, resulting from the unequal distribution of the positive and negative charges on the two sides of the membrane.

Mitchell's hypothesis provided an easy explanation for the action of many compounds of different chemical structures that uncoupled respiration from the production of ATP: they were simply catalysing the equilibration of protons and other ions across the membrane, and therefore dissipating the gradient of protons (and other ions). The following years were devoted to finding evidence to support the model. This involved demonstrating the release of protons by mitochondria during respiration (Mitchell and Moyle 1965) and quantifying the relation between the numbers of electrons transported in the respiratory chain, protons exported from mitochondria and ATP molecules produced (Mitchell and Moyle 1967). Experiments by others seemed to show the absence of a significant pH gradient (Chance and Mela 1966a, b, c; Slater 1967) or raised doubts about other parts of Mitchell's model (Tager *et al* 1966), but Mitchell criticised these experiments (Mitchell *et al* 1968), and also explained why the pH gradient was small (Mitchell 1967).

The strongest experimental arguments in favour of Mitchell's model came from photosynthesis. As early as 1963, Andre Jagendorf showed that vesicles from chloroplasts were able to accumulate energy derived from light (Hind and Jagendorf 1963) in the form – as shown three years later – of a gradient of protons (Jagendorf and Uribe 1966): the artificial generation of a proton gradient across the membrane was sufficient to trigger the synthesis of ATP from ADP and phosphate. The reality of the electrical component of the protonmotive force was also first demonstrated in plants (Junge 2004). A decade later, Dieter Oesterhelt and Walther Stoeckenius (1973) discovered that the purple membrane of *Halobacterium halobium* is able to make use of molecules of bacteriorhodopsin to capture the energy of light by generating a gradient of protons across the bacterial membrane. Efraim Racker and Stoeckenius then created artificial vesicles combining ATPase from bovine heart and bacteriorhodopsin from *Halobacterium* that synthesised ATP when submitted to light: a wonderful confirmation of the model of Mitchell (Racker and Stoeckenius 1974; Allchin 1996). This debt to photosynthesis was not fully acknowledged. Photosynthesis is a particular field of research – the study of plants was until recent years totally separated from the study of animals and microorganisms –, a field in which the direct influence of physicists and physical techniques is strong; an additional reason for biochemists to neglect what happened in this field of research (Junge 2004).

3. The present chemiosmotic model is not the model proposed by Mitchell in 1961

This brief historical sketch should not mask the fact that the model proposed by Mitchell in 1961 was very different from the one we know today. In the 1961 model of Mitchell, the protons generated by the respiratory chain accumulated inside the mitochondria, and not outside as was shown later. The redox loops proposed by Mitchell were also wrong. More seriously, as noticed by many observers (Malmström 2000; Prebble 2002), both the export of protons from the mitochondria and the synthesis of ATP were seen by Mitchell as direct consequences of the orientation of chemical reactions. In the present model, the movement of protons is also due to their being pumped through the membrane, a consequence of the transconformation of the

proteins involved in electron transport (Wikström 1977; Verkhovsky *et al* 1999). And in a similar way, the protons do not directly displace the ADP-ATP equilibrium, but alter the binding of ATP through a complex transconformation of the catalytic F1 part of ATPase; this leads to the release of ATP in the mitochondrial matrix.

These differences should not be thought of as mere 'mechanistic details' of an otherwise ('globally') correct model. As formulated, the *vectorial chemistry* model of Mitchell was precise. It is no longer true. What remains true is his intuition of the production of a proton gradient dependent upon the existence of an impermeable barrier – the cell or organelle membrane. We are better armed now to appreciate the true contribution of R J P Williams to the elaboration of the model. He shared with Mitchell the conviction that the production of ATP during respiration was the direct consequence of an increase in proton concentration. But he saw this effect as local, taking place within the hydrophobic membrane (Williams 1961, 1962). Although local concentrations of protons may occur, Williams did not see, or emphasise, the importance of the barrier formed by the organelle membrane. Therefore, there were clearly new, original aspects in the model of Mitchell that are still true today and were not present in Williams's model. This does not touch on the question of whether Mitchell was right to discuss the issue extensively with Williams in 1960 without laying bare his own thinking.

There was a second original aspect in the proposal of Mitchell, in his 1961 article as well as in his previous contribution to the mechanisms of bacterial transport of metabolites: the idea that some enzymes are precisely positioned within the cellular or organelle membrane (Mitchell 1957). Such an hypothesis was opposed to the dominant model of the lipid bilayer in which proteins only stuck to the membrane surface. Two developments therefore had a major, under-appreciated role in the acceptance of Mitchell's hypothesis. The first was the progressive demonstration by electron microscopy (Branton 1966; Henderson and Unwin 1975) and biochemical and biophysical methods (Lenard and Singer 1966) that proteins can be 'fully integral' components of membranes and interact with ligands on both sides of the membranes. It was demonstrated that these proteins – or protein complexes as in the case of the respiratory and photosynthetic chains – are mobile within the plane of the membrane, as proposed in the famous Singer and Nicolson article of 1972 (Singer and Nicolson 1972). This permanent movement of protein complexes within the plane of the membrane opposed the existence of any stable structural relation between the components of the redox chain and ATPase. The connection between the two systems did not take place at a particular place in the mitochondrial membrane, but at a global mitochondrial level. It supported the hypothesis that what was important was the gradient of protons, not the concentration of protons at a particular place.

The second development that favoured the eventual acceptance of Mitchell's hypothesis was a clearer understanding of protein structure. This hinted at the possibility that ionic effects, including the displacement of protons and electrons, could be the result of protein transconformation. The role of protons in protein conformation was demonstrated by the careful studies of Max Perutz on haemoglobin at the end of the 1960s. More generally, the discovery of the allosteric properties of proteins and enzymes gave proteins powers hitherto limited by their conception as simple chemical catalysts. Paul Boyer was the first to propose a role for transconformation in oxidative phosphorylation as early as 1964 (Boyer *et al* 1973; Boyer 1975a, b). Mitchell was violently opposed to this hypothesis – rapidly adopted by all those who had abandoned hope of discovering the mysterious high-energy intermediate – because he considered it to be too vague. He answered by reasserting his own model (Mitchell 1974). Paradoxically, the transconformational model is now a full part of the chemiosmotic model, and one of its strongest supports. The progressive understanding of proteins as nanomachines provided arguments in support of the theory of Mitchell.

Similarly, Mitchell pointed out that the energy stored in the proton gradient could be used for purposes other than ATP production – for example, for the movement of a flagellum in a bacterium (Mitchell 1972). This was a brilliant idea and is well accepted today. But the mechanism proposed by Mitchell, the electrophoretic mechanism, was different from the one that was finally retained (Manson *et al* 1977). Once again, the intuition of Mitchell was right, but many years were necessary for a proper mechanism to be proposed.

4. What does this complex story tell us about science?

The first lesson, which will be no surprise for the readers of this series in the *Journal of Biosciences*, is that, in biology especially, scientific knowledge is constructed progressively. It is rare for a theory that is eventually judged successful to emerge abruptly in the form that will finally be accepted. More often, as in the case of the chemiosmotic model, a theory is built 'as it goes along'. In the process it incorporates different observations and becomes more and more acceptable to the whole community. It can be difficult at the end to appreciate the contribution of the original hypothesis to the final product.

What made the centrality of Mitchell's contributions stand out to many actors in the field, and equally many outside it, was his insistence that the chemiosmotic model was

proposed in its mature form from the beginning (Mitchell 1979). By opposing new observations such as the pumping of protons by components of the respiratory chain (Moyle and Mitchell 1978), Mitchell took the risk of being wrong. But, by doing so, he made it less likely that his contribution would get diluted by that of many others. His personal style of work and interaction earned him a certain reputation that was difficult to erase when the time came for a consensus. He presented his work as a wonderful illustration of Karl Popper's emphasis on the importance of theories and their falsification for the progress of science. This was clear support at a time when Popper's philosophy was becoming widely accepted among scientists (Mitchell 1977).

The traditional approach of philosophers of science when they outline the criteria for the acceptance of a new theory is to highlight its parsimony and the way it generates new *crucial* experiments (Weber 2005). A second lesson for us to draw is that these criteria are clearly insufficient to understand why the chemiosmotic model triumphed in the mid-1970s. Many challenges to the chemiosmotic theory had accumulated by then. Among them were issues concerning the stoichiometry of the reactions, the existence of active proton pumping processes and the indirect role of protons in ATP formation. All of them could have made the acceptance of the chemiosmotic model more difficult. But they did not. The elegant hypothesis of the Q (ubiquinone) cycle proposed by Mitchell in 1975, a pure *biochemical* hypothesis, probably helped to convince the community of biochemists of Mitchell's credibility: he was no longer an outsider (Mitchell 1975a, b).

The initiative of Racker to give the field of bioenergetics a higher profile by showing what was shared by all protagonists instead of focusing on disputes and conflicts was very important. It led, after a long and difficult negotiation, to the publication of a multi-author review in the *Annual Review of Biochemistry*. Bringing together the major actors in the field, it was centred around a discussion of the chemiosmotic model of Peter Mitchell. Although not all participants agreed on the value of this model, it obviously placed it and Mitchell alone under the spotlight (Boyer *et al* 1977). It was, directly or indirectly, a crucial step toward the award, one year later, of the Nobel Prize to Mitchell alone.

But most important for the acceptance of the chemiosmotic model was its progressive wedding to the mechanistic vision of proteins within membranes. Membranes, and the proteins embedded in them, became a major focus of interest at the beginning of the 1970s in immunology, in the study of differentiation and development, and in understanding the formation of tumours (Morange 2000). The resulting rapid progress made in the isolation and characterisation of membrane proteins and the reconstitution of active vesicles go a long way to explaining the full recognition of Mitchell's contribution, more so than the patient and painful experimental work he did to provide arguments in favour of his model. Particularly important in this process was the reconstitution work that Racker initiated at the beginning of the 1960s, which generated thirty articles and, as we described before, became one of the most compelling factors working in favour of Mitchell's model. To isolate the active molecules, to characterise their properties and then to reconstitute the global function by putting these molecules together is a way of proceeding that is characteristic of biochemistry (*see*, for instance, one step in this work: Kagawa and Racker 1971). Therefore, the long evolution, i.e. the characterisation of transmembrane proteins and the progressive appreciation of their multiple powers, and *events* such as the description of the Q cycle, the discovery that the proton gradient can generate the movement of bacterial flagella and can produce heat in the specifically adapted mitochondria of the brown fat tissue, created a kind of resonance favourable to the award of the Nobel Prize to Peter Mitchell.

What lessons for the present does this successful saga teach us? Despite the achievements of Mitchell, Glynn cannot be seen as a new way to do research: the influence it had despite its isolation was obviously due to the exceptional personality of Peter Mitchell. The financial support was problematic: its functioning was only made possible through investment by Mitchell's family. The Glynn Research Institute accumulated debts, not profits (Prebble and Weber 2003). Mitchell permanently faced financial difficulties, and fundraising occupied more and more of his time. After his death, and despite the efforts of his successor, Peter Rich, Glynn was closed in 1996, four years later.

The difficulties encountered by Mitchell also underline once more how much effort interdisciplinarity requires. Mitchell's intuitions came from his familiarity with physical chemical studies of membrane transport (Robinson 1997). This field of research belonged to physiology. Because of the 'space' that it accorded to formal representations, it was different from the field of conventional biochemistry. For most researchers who were looking for the hypothetical energy-rich intermediate, the equations written by Mitchell made no sense and had no explanatory value. The explanation of a phenomenon required the description of a mechanism by which this phenomenon could be generated. This emphasis on mechanisms is a characteristic of a large part of present-day biology (Darden 2006), and it clearly distinguishes this discipline from others. Most biologists accepted the theory only once an understandable model was at hand: only after the abstract electrochemical potential was replaced by a reversible mechanistic action of protons on the structure of proteins. The electrochemical potential was for them at best a way to summarise what happened, not the final explanation of the phenomenon. The same was true of

proticity, the power coming from the unequal distribution of protons, something equivalent to electricity for Mitchell. For biochemists, what was important were individual protons, the action of which resulted from their interaction with proteins. Therefore, the acceptance of the chemiosmotic theory can hardly be seen as the recognition of the place of theory in biological thinking. Whether this place will increase in the future is yet another question.

Acknowledgements

I am indebted to David Marsh for critical reading of the manuscript, and to the reviewers who helped me to discard some errors, and to add information which, I hope, helps to improve this article.

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*e*Publication: 7 December 2007