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# Contrasting Approaches to a Biological Problem: Paul Boyer, Peter Mitchell and the Mechanism of the ATP Synthase, 1961–1985

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**Abstract.** Attempts to solve the puzzling problem of oxidative phosphorylation led to four very different hypotheses each of which suggested a different view of the ATP synthase, the phosphorylating enzyme. During the 1960s and 1970s evidence began to accumulate which rendered Peter Mitchell's chemiosmotic hypothesis, the novel part of which was the proton translocating ATP synthase (ATPase), a plausible explanation. The conformational hypothesis of Paul Boyer implied an enzyme where ATP synthesis was driven by the energy of conformational changes in the respiratory proteins. This was finally abandoned as an explanation of the overall process. Nevertheless the conformational understanding of the enzyme became an acceptable proposal during the early 1970s and eventually led Boyer to a view of the enzyme that incorporated both hypotheses. The correspondence between Mitchell and Boyer, both Nobel laureates, exposes their different approaches to both this enzyme and to the hypotheses of oxidative phosphorylation and illuminates a key step in the development of bioenergetics. In particular Boyer was suspicious of proton gradients, because he could not envisage a chemical mechanism for the synthesis of ATP, while Mitchell distrusted conformational arguments because he believed the proton must act vectorially at the active site of the enzyme. This resulted in two different views of the mechanisms operating in this enzyme. Ultimately while Boyer was able to marry the two approaches, Mitchell retained his insistence on the role of the proton at the active site and was thus unable to give significance to Boyer's conformational ideas. The underlying issues in this debate are discussed particularly with reference to the differing styles of Boyer and Mitchell and the influence of molecular biology, especially the development of protein technology.

**Keywords:** ATPase, ATP synthase, Boyer, Mitchell, Oxidative phosphorylation, Phosphorylation, Styles of science

## Introduction

In the discipline of biochemistry, few areas have produced as much controversy or debate as oxidative phosphorylation, the process whereby the

energy from the oxidation of foodstuffs is conserved in the synthesis of ATP, often seen as the energy currency of the cell. This was especially so over the three decades after about 1955. Central to these debates, among others, were Peter Mitchell (1920–1992), originally a microbial biochemist, and Paul Boyer (1918–) originally a chemist, both Nobel laureates.[1] By 1964, at least four hypotheses had been advanced to explain the basic process. The situation was not resolved until the end of the 1970s when the majority of workers accepted that the link between oxidation and phosphorylation was an electrochemical potential gradient of protons, formed by oxidation and dissipated by ATP synthesis, the chemiosmotic hypothesis of Peter Mitchell. Boyer concerned himself initially with the chemical hypothesis for the mechanism of oxidative phosphorylation but then developed a new approach, the conformational hypothesis. Although this theory has been seen as an error in retrospect, it nevertheless led to an understanding of the ATP synthase.[2] Later he concentrated on investigating the mechanism of the ATP synthase itself, one of the most sophisticated enzymes known, to which he successfully applied his conformational ideas. Indeed, the hypotheses for explaining oxidative phosphorylation dictated the way the ATP synthase[3] was understood.

First, I examine the history of the understanding of the ATPase initially through the four theories of oxidative phosphorylation which generated contrasting views of the enzyme. The supporting evidence is summarised in order to perceive the issues raised by the limitations of experimentation. The design of experiments which were not open to alternative interpretations proved a major problem. As Boyer commented after more than a decade of intensive investigation in many laboratories, "firm evidence about oxidative phosphorylation, other than that the remarkable process occurs, is disappointingly meagre."[4] Such a situation encouraged the theoretical approach to the understanding of oxidative phosphorylation.

[1] Boyer shared the 1997 Nobel Prize in Chemistry for the "elucidation of the enzymatic mechanism underlying the synthesis of adenosine triphosphate (ATP)." For short autobiographies, see Boyer, 1981, 1998a. Mitchell was awarded the 1978 Nobel Prize in chemistry for his "contribution to the understanding of biological energy transfer through the formulation of the chemiosmotic theory." For a short biography, see Slater, 1994 and for a full biography, see Prebble and Weber, 2003.

[2] See Allchin, 2002.

[3] The ATP synthase is the central enzyme of oxidative phosphorylation and catalyzes the reaction: ADP + phosphate = ATP + water. The reaction requires energy normally provided by respiration (ADP = adenosine diphosphate, ATP = adenosine triphosphate). The enzyme synthesizes most of the ATP in aerobic cells and, in eukaryotic cells, is found in the mitochondrion. It is also known as the ATPase since it was discovered as an enzyme which broke down ATP.

[4] Boyer, 1968, see p. 229.

The second part of the paper examines the correspondence between Mitchell and Boyer which reflects both the successes and problems of the period leading up to an understanding of the enzyme and of oxidative phosphorylation in general. In these letters both men develop their views on the enzyme and inform each other in their letters between the years 1973 and 1987, although there is a limited correspondence before that period.[5] The transition from Mitchell's view to the more comprehensive approach of Boyer marks one of the defining advances in the twentieth century understanding of the process of oxidative phosphorylation. Their correspondence provides an insight into the approach to biological science of both men. This leads to a consideration of the reasons for the conflicting views of the two.

## The ATP Synthase as Seen in the Hypotheses for the Mechanism of Oxidative Phosphorylation

Although mitochondria were known to possess an ATP hydrolysing enzyme from around 1950, the understanding of the enzyme up to the mid-1970s was framed by the four[6] main hypotheses for the mechanism of oxidative phosphorylation. E.C. (Bill) Slater (1917–) proposed the chemical hypothesis, Robert J.P. Williams (1926–) proposed a proton-based hypothesis, while Mitchell put forward his chemiosmotic hypothesis and Boyer the conformational hypothesis. Each formulation generated a different view of the ATP synthase in the mitochondrion (and the chloroplast).[7]

The initial inspiration for thinking on biochemical phosphorylation related to oxidation arose out of the earlier development of

[5] The extant correspondence begins in 1961 and ends in 1987 five years before Mitchell died. While discussion of the mechanism of oxidative phosphorylation and more particularly the mechanism of the ATP synthase is the main subject of the correspondence, there is a second theme, only briefly considered here, concerned with seeking agreement for the collaborative 1977 review (Boyer et al., 1977). The latter issue is discussed in detail in Prebble and Weber, 2003, pp. 195–208. The letters are held in the P.D. Mitchell Archive in the library of Cambridge University, UK, but these have been supplemented by letters kindly provided by Professor Boyer.

[6] I have considered here the four proposals for the mechanism of oxidative phosphorylation which were those with a long life in the literature and which were seriously considered by significant groups of workers in the field. There were other hypotheses which could also be considered, for example, the importance attached to the conformational changes observable in the structure of the mitochondria themselves by David Green. See Green and Baum, 1970.

[7] The enzyme discussed here operates in mitochondrial and enteric bacterial oxidative phosphorylation and in plant chloroplast photophosphorylation. While the early work concentrated on mitochondria, usually from mammals, studies on chloroplast and bacterial phosphorylation made a significant contribution from the mid-1960s.

intermediary metabolism,[8] which was central to the formation of biochemistry as a discipline. The idea of ''an unbroken account of the chemical reactions connecting the foodstuffs that enter an organism with the end products that leave it'' was the theoretical vision that drove the biochemists during the early part of the twentieth century.[9] The pathway for glucose breakdown, the glycolytic pathway, the urea cycle and the citric acid (Krebs) cycle formulated in the 1930s provided a basis for a 'metabolic map.' A second phase of investigations into intermediary metabolism involved the elucidation of the reaction mechanisms for the individual reactions that made up the metabolic pathways.[10] In 1939, Otto Warburg (1883–1970) and his colleagues reported on the mechanism of a key reaction in glycolysis, glyceraldehyde phosphate oxidation, a study which served not only to clarify a step in the glycolytic pathway but also brought together ideas on oxidation and phosphorylation.[11] It was this ''complete description of a biological coupling'' between triose phosphate (glyceraldehyde phosphate) oxidation and the uptake of inorganic phosphate that Kalckar believed would provide ''a clarification of other energetic couplings.''[12] The key elements of this work, were firstly that the oxidation step was coupled to a phosphorylation of the substrate with inorganic phosphate. Secondly, the phosphate was transferred to ADP to form ATP. Since the respiratory chain (the oxidation process) could function well in the absence of inorganic phosphate, Slater found it necessary to postulate ''a high energy'' chemical intermediate which was phosphorylated. Thus Warburg's study provided a basis for Slater's chemical hypothesis but, in addition, substantially influenced Boyer's ideas on conformational coupling. In his paper introducing conformational coupling Boyer wrote, ''for many years the only even partially satisfactory understanding of how an oxidative reaction is coupled to ATP synthesis has been the sequence catalyzed by glyceraldehyde-3-phosphate dehydrogenase and 3-phosphoglycerate kinase.''[13]

Slater's proposal,[14] and its several variants, became known as the chemical theory and dominated thinking about oxidative phosphorylation

[8] See Prebble, 2010.

[9] Holmes, 1991, see p. 35.

[10] Holmes, 1992, see pp. 77 and 81.

[11] Warburg and Christian, 1939; Negelein and Brömel, 1939. Warburg's mechanism was later modified, see Racker, 1965.

[12] Kalckar, 1941. See p. 131 but also pp. 122–131.

[13] Boyer, 1965, see p. 994.

[14] Note that Fritz Lipmann (1899–1986) put forward a simple view of the mechanism on which Slater built substantially (Lipmann, 1946).

from 1953 into the 1960s and remained in contention until the early 1970s. Based on Warburg's study, the proposal viewed the ATP synthase as an enzyme transferring phosphate from a hypothetical intermediate (formed by respiratory activity) to ADP forming ATP.[15] Thus it had a firm experimental basis in the biochemical understanding of the 1940s and 1950s. By 1973, Slater regarded his proposals as lacking usefulness or credibility.[16]

In 1961 the Oxford inorganic chemist, R.J.P. Williams, described how a proton might act in phosphorylation based on the chemistry of polyphosphate formation.[17] Experimental evidence for or against this proposal proved almost impossible to obtain in the 1960s and 1970s but the hypothesis was a subject for serious consideration while the mechanism of oxidative phosphorylation remained obscure. This view provided a different approach to the nature of the ATP synthase and involved the proton acting essentially along and within the membrane. The restriction of the proton to the membrane phase was often seen as its most significant element. The strong superficial similarities between Mitchell's and Williams' theories led to strained relations between the two but did little to advance the understanding of the ATPase.[18]

Mitchell's appreciation of the ATP synthase was defined by his chemiosmotic hypothesis published in 1961. Indeed, he regarded the really novel aspect of his proposal as the ATPase (ATP synthase) driven by a pH gradient across the mitochondrial membrane created by the respiratory system.[19] The theory can be best understood in two parts. First, the respiratory chain in the inner mitochondrial membrane creates a pH gradient across the membrane and an accompanying electric potential. Second, the gradient and potential drive ATP synthesis by the ATP synthase. At this stage Mitchell assumed that the enzyme worked by the removal of hydroxyl and hydrogen ions (the components of

[15] Slater, 1953. It should be noted that Boyer felt that this paper simply reflected the thinking of biochemists at that time (Interview Boyer 11 December 1993). The value of the proposal lay in its stimulation of research and it was very widely quoted in the 1950s and 1960s. See Chance and Williams, 1956 (particularly pp. 97–100) for examples of development of the chemical proposal.

[16] After the failure of an extensive search for the predicted intermediate in many laboratories, Slater commented in a lecture to the Biochemical Society that the high-energy intermediates of the chemical proposals probably do not exist (Slater, 1974, see p. 1153).

[17] Williams, 1961. A brief preview appeared two years earlier.

[18] Williams' and Mitchell's theories and the extensive correspondence between Mitchell and Williams have been analyzed in Weber and Prebble, 2006.

[19] Mitchell, 1961. Mitchell had given a hint of his thinking at a Stockholm conference in 1960.

water) from phosphate and ADP to form ATP, induced by the pH gradient and membrane potential. The hypothesis was welcomed by Boyer "It is refreshing to see a different approach to the possible mechanism of coupling of phosphorylation to electron transport as given in your paper. Certainly we have been too limited in our thinking."[20] Such a comment should be taken at face value; it did not imply support for the proposal and later he commented that Mitchell presented his ideas in language which did not correlate with that of workers in the field at that time. Indeed for some time the chemistry employed by Mitchell obscured the significance of his proposals for Boyer who felt that this was a reason why initially it was largely ignored.[21]

Mitchell's proposals and particularly those relating to the ATP synthase were derived from his speculative consideration of membrane-bound enzymes.[22] Thus, it is not surprising that there were a number of details which on experimental investigation proved to need revision. The most significant of these was the fact that the original proposal proved to have the wrong polarity for the membrane. A revised version of the hypothesis, which was then being regarded more as a curiosity than a plausible explanation of oxidative phosphorylation, was therefore required. In 1966, this led to a substantial paper which was too long for publication in his chosen journal, *Biological Reviews* where he published an abridged version; the original version was published privately.[23] This new version of the chemiosmotic hypothesis was essentially the one that is now current, but Mitchell included several detailed mechanisms for both the respiratory chain and the ATPase. These were mostly abandoned at an early stage because of inconsistency with new experimental results or criticism from other workers often on chemical grounds. Indeed it was this speculative aspect of many of his proposals that caused some scientists initially to reject his proposals.

The ATP synthase itself was now seen as a proton-translocating enzyme and, as Mitchell pointed out to Boyer, "the synthesis [of ATP] actually depends upon the flow of protons from one aqueous phase to another through the ATPase system of the membrane."[24] A significant

[20] Letter, Boyer to Mitchell, 18 December 1961. P.D. Mitchell archive file G 76.

[21] Interview with Boyer, 11 December 1993.

[22] I have explored in some detail both the philosophical and experimental background to the hypothesis in Prebble, 2001.

[23] Mitchell, 1966a. He published the full version from his private institute, Glynn, which became known as the first 'Grey Book' (Mitchell, 1966b). It was widely circulated among those working in the field. Chapter III was devoted to the proton translocating ATPase system.

[24] Letter, Mitchell to Boyer, 6 October 1966. P.D. Mitchell archive file G 76.

part of the mechanism was that the proton was involved at the catalytic site for ATP synthesis.[25] At this stage Mitchell seems to have had three guiding principles to his proposals for the proton-translocating ATPase. Firstly they would only be acceptable to the bioenergetics community if a mechanism for translocation of the proton could be found, secondly any mechanism must involve the proton in catalysis at the enzyme active site and thirdly the mechanism proposed should be quantitative. Boyer's comment on the possibility of the proton acting directly at the catalytic site was: ''As a chemist, I find myself continually searching for how a proton can actually induce bond formation. Definitive suggestions as to how such chemistry can be coupled to the flow of protons from one aqueous phase to another have not been evident to me'' Thus Boyer indicated his inability to accept the central mechanism for ATP synthesis advanced by Mitchell. He added: ''Even with a 'chemiosmotic' driving force, ATP synthesis would need a chemical basis. To present the 'chemiosmotic' hypothesis as distinct from chemical mechanisms thus does not appear useful.''[26] Criticisms of Mitchell's chemistry would become much stronger in the 1970s.

Thus the connection between ATP synthesis and respiration was not a chemical compound as Slater had suggested, nor a local high concentration of protons in the membrane as Williams had suggested. Mitchell saw the connection as a pH gradient and membrane potential across the membrane and the ATPase as a proton translocating enzyme.

In the mid-1960s, when Boyer was exploring candidates for the elusive high energy intermediate of the chemical theory,[27] he proposed the fourth major hypothesis for oxidative phosphorylation. A guiding theme in Boyer's work was the need to describe all of the bonds formed and broken in the process. Thus, the energy of oxidation might be conserved as an associated formation of an acyl-S bond and that such a bond might generate a phosphohistidine group where the phosphate would then be transferred to ADP to form ATP. This concept could be seen as a part of the search for a high-energy intermediate as predicted in the chemical hypothesis. In order to link the formation of the acyl-S bond with oxidation in the respiratory chain, Boyer thought that changes in protein conformation of respiratory intermediates would be

[25] Mitchell's suggested mechanism of translocation, which now took account of the work of Fernández-Morán and Racker (see particularly Fig. 1), introduced an unidentified intermediate – a step which caused many in the field to feel there was now not much difference from the chemical theory where an unidentified intermediate had been the major problem with the proposal.

[26] Letter, Boyer to Mitchell, 9 November 1966. P.D. Mitchell archive file G 76.

[27] See Allchin, 1997, 2002.

necessary. Such speculations were supported by work on the phosphorylation associated with the citric acid cycle but as Boyer's 1965 paper shows, the glycolytic phosphorylation was foundational.[28] The hypothesis also drew inspiration from the fact that a protein conformational change was associated with ATP hydrolysis in muscle contraction. The proposal was welcomed by several workers, particularly those who were pursuing large scale changes in mitochondrial conformation associated with ATP hydrolysis.[29]

Boyer did not develop his views until the early 1970s when it occurred to him that an oxygen exchange measured between phosphate and water could be interpreted in terms of the use of energy from respiration to release ATP from the ATP synthase (rather than to synthesize the covalent bond in ATP). Such a view implied a conformational change in the enzyme itself as the means of supplying energy for ATP release. This conceptual advance was based on limited evidence and the *Journal of Biological Chemistry* rejected his paper. It was then published in the *Proceedings of the National Academy of Sciences* in 1973.[30] Thus the conformational theory saw the ATP synthase as synthesising ATP on the surface of the enzyme which then underwent an energy-dependent conformational change bringing about ATP release.[31] Such a view saw the source of energy as the conformational changes in the respiratory chain during oxidation but found no place for high energy intermediates (chemical theory), membrane protons (Williams, theory) or transmembrane proton potential gradients (chemiosmotic theory). Later Boyer made proposals to accommodate a proton electrochemical gradient.

The four hypotheses for the mechanism of oxidative phosphorylation reflect much of the development of the discipline of biochemistry itself, particularly its hybrid nature.[32] Slater's chemical proposals can be seen

[28] The original version of this theory was proposed in Boyer, 1965.

[29] In the 1960s, considerable interest was expressed in a view that oxidative phosphorylation was to be understood through conformational changes in the mitochondria themselves which could be observed with electron microscopy. In general these changes were later seen as an expression of osmotic events and ion movements in the particles. This type of thinking is exemplified in Green and Baum, 1970.

[30] Boyer et al., 1973.

[31] See Boyer, 1981, pp. 234–235 for a personal comment on these events.

[32] This was explored from an institutional point of view by Kohler, 1982. He found medicine, chemistry and biology all contributed to the development of the discipline but in the United States, biology was the 'poor relation'. In the United Kingdom, particularly at Cambridge, there was a more open approach to the subject where 'general biochemistry', that is biochemistry which not only considered mammalian aspects but which also linked to microbiology and plant physiology, was developed (see pp. 73–89).

as an outcome of the investigative stream of intermediary metabolism which can be traced back to nineteenth century physiological chemistry.[33] Certainly the glycolytic pathway, which was one of the first really concrete results of the study of intermediary metabolism can be seen as providing the basis for the study and understanding of aerobic phosphorylation. When the weaknesses of Slater's approach began to be apparent around 1960, new approaches became desirable if only to widen thinking in the field and new hypotheses emerged. The development of biochemistry has drawn heavily on chemistry and perhaps it is not surprising that a concept directly drawn from inorganic chemistry was proposed by Williams. However, it was Boyer who, initially seeking the elusive intermediate of the chemical hypothesis, began to consider the possibilities raised by the developing understanding of the chemistry of proteins, in particular the possibilities raised by changes in protein conformation. The contribution of Mitchell highlights the importance of a biological approach to biochemical problems. His approach lacks the skills of the chemist and the appreciation of proteins but exploits the growing knowledge of biological membranes.

## Experimental Evidence

Distinguishing between the four proposals described was a major preoccupation of workers in the field during the 1960s and early 1970s. Definitive experimental evidence was not readily obtained. Thus the understanding of the synthesis of ATP in mitochondria (and also chloroplasts) remained somewhat confused. However, four lines of evidence significantly influenced conceptual thinking on the ATP synthase in the 1960s and early 1970s. First, starting in the 1950s, isotope exchange experiments by Boyer showed that the ATPase reaction (hydrolyzing ATP to ADP and inorganic phosphate) observable in mitochondria was reversible and that the enzyme was capable of acting as an ATP synthase.[34] There were several such 'exchange reactions' and they generated considerable information on the enzyme but they showed that the ATP synthase could be studied independently of respiration.[35] The exchange of the heavy isotope of oxygen ($^{18}O$) between

[33] Holmes, 1992.

[34] Boyer et al., 1954. These experiments were in part based on earlier work of Cohn, 1953.

[35] Boyer et al., 1966.

water and inorganic phosphate (an indicator of ATP synthesis) was not as sensitive to uncouplers as might have been expected. Since uncouplers were seen as blocking the energy supply to the enzyme, this later raised questions about the possibility of synthesis of ATP without energy and, as noted above, inspired Boyer to propose the revised conformational theory in the 1970s.

Second, Efraim Racker (1913–1991) and his group at the Public Health Research Institute of the City of New York and later at Cornell University isolated the ATP synthase in 1960 and showed that the isolated enzyme was responsible for phosphorylation of ADP.[36] This work gave a basis for understanding the enzyme and in due course led to a basic description of its properties. In particular Racker's group interpreted the earlier discovery of Humberto Fernández-Morán (1924–1999) an electron microscopist then working in the Massachusetts General Hospital, Boston and later Venezualan politician, who observed small particles attached to mitochondrial membranes (see Figure 1a).[37] Racker's work showed that the enzyme consisted of two parts, known as $F_1$ and $F_0$, the former containing the catalytic site for ATP synthesis.[38] These studies initiated a visual approach to the enzyme developed further by Racker's group (Figure 1b). Such studies provided the basis for understanding the enzyme but initially were interpreted in terms of any theory. Later, work supported the concept of a proton translocating ATPase (chemiosmotic theory).

Third, in 1966, work in the laboratory of André Jagendorf (1926–) at John Hopkins University showed that the application of a proton gradient to broken chloroplasts which possess an energy-dependent ATP synthase, could result in the synthesis of ATP in the dark without other energy source.[39] These experiments were confirmed in other laboratories. Such evidence supported the chemiosmotic view of the enzyme but for a while the strength of these results was muted by the failure to obtain similar results with the mitochondrial enzyme. It should be noted that Mitchell did publish results of experiments in 1966 in which he claimed ATP synthesis in response to a proton gradient[40]

[36] Pullman et al., 1960; Penefsky et al., 1960. A preliminary account of this work appeared two years earlier.

[37] Fernández-Morán, 1962.

[38] Kagawa and Racker, 1966. Note that the results of these experiments were communicated before publication to Mitchell. The significance of this work is explored by Bechtel and Abrahamsen, 2007 (see p. 21 f.) who saw these structural–functional relationships as the outcome of the impact of cell biology on biochemistry.

[39] Jagendorf and Uribe, 1966.

[40] Reid et al., 1966.

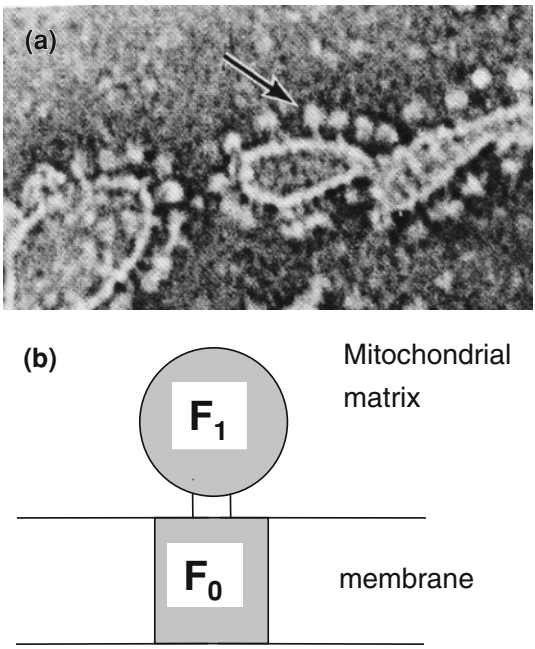



*Figure 1*. The structure and location of the ATP synthase on mitochondrial inner membranes. (a) An electron micrograph of negatively stained fragments of mitochondrial membranes (later known to be the inner membrane) showing the spherical particles attached to the membrane by a small stalk. (Photograph provided by the late Professor Fernández-Morán and published in *Journal of Cell Biology* 22, © Fernández-Morán et al., 1964, part of Fig. 5.) (b) A diagram showing the relationship of the major parts of the ATP synthase following the work of Racker and others which provided an interpretation of the electron micrographs above. Racker's group showed that the sphere ($F_1$) had the properties of the ATP synthase. It was concluded that $F_1$ was attached to a complex embedded in the membrane named $F_0$ which was shown to transmit protons across the membrane

but the amounts were small and they carried little weight with other workers. Boyer pointed out to him that in addition to Mitchell's own interpretation of his experiments, other interpretations were possible. "It could represent reversal of ATP driven ion transport. It could represent a transient $P_i$ = ATP exchange reaction… mitochondria have a considerable ATP pool, and exchange as opposed to net synthesis will be difficult to rule out in your experiments."[41] Such difficulties in assessing the meaning of experiments were not infrequent in the field.

Evidence for proton translocation was provided by Mitchell and his research associate, Jennifer Moyle, who demonstrated that two protons

[41] Letter, Boyer to Mitchell, 9 November 1966. P.D. Mitchell archive file G 76.

crossed the membrane when ATP was hydrolysed. They also showed that respiration transferred six protons for every oxygen reduced so that the P/O ratio[42] was 3, the accepted figure at that time. Impressive as these results might seem, there was some scepticism about them and later in the 1980s, after a major controversy, they were amended. Although various experiments appeared to confirm that the enzyme was a proton-translocating one, other interpretations were still possible. Racker commented: "Thus it cannot be decided on the basis of such experiments whether a proton or an ion gradient is on the direct pathway of energy production."[43]

Such comments underline the fact that so much experimentation in this period, including Mitchell's experiment discussed above, although progressively providing information, was frequently capable of other interpretations. In this case, for example, was the proton motive force creating the high energy intermediate of the chemical theorists or was it acting directly on the ATPase enzyme? With a complex mitochondrial system such as oxidative phosphorylation, it proved very difficult to design experiments which demonstrated unambiguously particular aspects of the mechanism. Thus Mitchell's attempt to show proton-driven ATP synthesis could be interpreted in several other ways. This was a common problem in this field and often led to clashes between workers. A similar problem has been described in the study of protein synthesis, also a complex system with many components.[44] Such problems are often overcome, as in this case, and also in elucidating the mechanism of protein synthesis, by separating out the components of the system.

For oxidative phosphorylation, perhaps the first successful step of this type was an experiment in which the isolated ATP synthesizing enzyme was combined with a protein capable of producing a proton electrochemical gradient in a vesicle. Thus the fourth line of evidence, provided by Racker and Stoeckenius, was a much less ambiguous demonstration of proton-driven ATP synthesis that proved highly influential among biochemists but not necessarily all bioenergeticists. These workers combined in a lipid vesicle the isolated ATP synthase enzyme as prepared by Racker and the bacterial protein bacteriorhodopsin (as prepared by Walther Stoeckenius 1921–). In the light this

[42] The P/O ratio is the ratio of the number of phosphorylations (molecules of ATP synthesised) to the number of atoms of oxygen reduced by the respiratory chain.

[43] Racker, 1970, see p. 134.

[44] See Rheinberger, 1997, p. 67. The author notes, in a study of in vitro protein synthesis where similar problems were encountered, that "the main experimental signal… was not a meaningful signal in itself".

system created a proton gradient across the vesicular membrane leading to ATP synthesis by the synthase. Thus this simpler system provided strong evidence supporting Mitchell's notion of a proton-translocating ATP synthase driven by the proton motive force.[45]

## Correspondence: Conformational Changes in Proteins

It was against the background of growing experimental evidence for Mitchell's chemiosmotic hypothesis (some of it open to alternative interpretations) and limited rather indirect evidence for Boyer's conformational theory that regular correspondence between the two began in 1973. At the time some workers supported Boyer, others Mitchell. Indeed, a number of workers saw Boyer's proposals as a revised version of the chemical hypothesis and after the demise of the latter at the beginning of the 1970s, they were attracted to Boyer's views rather than the ion gradient proposals of Mitchell. Mitchell on the other hand found the conformational approach unattractive particularly as it did not involve the proton directly in catalysis and as it did not appear to provide for stoichiometric relationships[46] with electron transport although he agreed that conformational changes in the ATPase would be expected. As he explained to Boyer:

> I agree with your notion concerning the probable involvement of conformational changes in the action of the ATPase during ATP synthesis – but… my view differs from yours in that I regard the conformational changes as a result of cooperative interactions within the enzyme-substrate complex between $H^+$ ions that pass through the complex under the influence of a proton-motive force, and ATP, ADP, $P_i$ and $H_2O$, the relative movements of which are obligatorily involved in the overall process catalysed by the enzyme.[47]

In his reply Boyer summarised his own position at this point. "I feel that protein–protein interaction merits additional consideration [conformational theory], but clearly likewise feel that your valuable concepts of proton motive force represents an interesting alternative."[48] In fact

[45] Racker and Stoeckenius, 1974. It should be noted that in discussion in 1991, Mitchell felt that this experiment, while proving persuasive with biochemists generally, did not prove as convincing to those actively working in the field.

[46] This refers to numerical relationships between chemical entities, for example the key stoichiometric relationship in oxidative phosphorylation is the number of phosphorylations (ATP molecules synthesised) to the number of atoms of oxygen consumed, the P/O ratio.

[47] Letter, Mitchell to Boyer, 25 June 1973. P.D. Mitchell archive file G 76.

[48] Letter, Boyer to Mitchell, 20 July 1973. P.D. Mitchell archive file G 76.

Boyer "had been unable to accept the concept advanced by Peter Mitchell… because I could not see a satisfactory way to use the proton motive force to make ATP."[49]

The two met at a Biochemical Society symposium in Bristol, England in September 1973. Boyer recalls that Mitchell presented his view of the enzyme published the following year, that involved a direct interaction of the proton with phosphate at the enzyme active site. In private discussion with Mitchell, Boyer explained "why I felt his suggestion was chemically unattractive. My comments had little or no impact."[50]

In subsequent correspondence they both defined their positions. Mitchell pointed out that in his theory

> there is no functional requirement for direct contact between components of the respiratory chain and the reversible ATPase… The work required for ADP phosphorylation is assumed to be done by the passage of protons through the ATPase under a proton motive force… Naturally, the proton translocation process, and the process of ADP phosphorylation involve cooperative movements within the ATPase enzyme system – as in many other enzyme systems – but these conformational movements are only incidental to the catalytic role of the ATPase system. They are not the initial source of the work transferred from the respiratory chain to the ATPase.[51]

In reply, Boyer pointed out that

> the major input [of energy] in the synthesis of ATP by oxidative phosphorylation occurs to bring about release of ATP from a tightly bound site. Our primary suggestion is that this release occurs by an energy-induced conformational change in the protein. Such conformational changes would not be only incidental to the catalytic role of the ATPase system – they would be the means by which the energy derived from oxidations was brought to bear on the catalytic site and thus on the formation of ATP. This would appear to be a chemically distinct mechanism from using protons directly at the catalytic site to preferentially protonate particular ionic forms of $P_i$, ADP and/or ATP.[52]

[49] Boyer, 1981, p. 234. This refers to Boyer's inability to envisage a chemical mechanism which would involve a proton.

[50] Boyer, 1981, p. 237.

[51] Letter, Mitchell to Boyer, 22 October 1973. P.D. Mitchell archive file G 76.

[52] Letter, Boyer to Mitchell, 28 November 1973. P.D. Mitchell archive file G 76.

He added that his results were not necessarily in conflict with Mitchell's concept that a potential gradient might be the energy conserving mechanism, an indication that Boyer's thinking was moving towards accommodating the chemiosmotic proposal within his view of the ATP synthase. In effect his core position now related to the mechanism of the enzyme but not how energy reached the enzyme. However, at the end of the letter he distinguishes his two proposals. The second is essentially his conformational theory and the first is his view of the ATPase mechanism:

> Our suggestions of conformational involvements in the energy transducing processes thus have two clear components. One is that conformational change is used to couple energy to the release of firmly bound ATP… The second aspect of conformational coupling is that the transfer of energy from the respiratory chain to the phosphorylating system may occur via protein–protein interaction and conformational changes.[53]

The existence of two separate conformational proposals appears to bring some confusion into the correspondence. Boyer argued that conformational changes *could* link the respiratory chain with phosphorylation but more importantly, he had evidence that the provision of energy for ATP synthesis probably proceeded through conformational change(s) whether or not his conformation hypothesis is valid. It is often not clear that Mitchell was distinguishing them adequately.

By 1974 Boyer was now canvassing, as an alternative to the transmission of energy to the ATPase by conformational changes, the transmission of energy by a proton motive force which would induce the conformational changes of the ATPase.

> The conformational coupling envisaged for ATP synthesis could be operative even if energy is transmitted by a potential gradient. For example, the gradient could produce protein conformational change in a manner akin to what Brit Chance has called a membrane Bohr effect. Were this true, vital aspects of both hypotheses would be correct.
>
> You note quite correctly in your letter that likely all catalytic events are accompanied by protein conformational changes. But the essential point in conformational coupling is that energy may be transmitted by such changes. This is the case in haemoglobin where binding of oxygen at one site causes small changes in positions of a number of atoms and stresses on a number of bonds to change the

[53] *Ibid.*

> affinity of oxygen at a distal site. In conformational coupling to ATP synthesis, the primary conformational stress or change might occur at some distance from the catalytic site for ATP formation.[54]

Boyer's reference to conformational changes in haemoglobin were probably prompted by the crystallographic work of Perutz demonstrating the structural changes in the molecule published four years earlier.[55]

Mitchell's reaction to such proposals can most readily be seen from a letter sent to Lars Ernster (1920–1998) one of the leading European bioenergeticists and copied to Boyer and others. Referring to Boyer's proposals Mitchell comments:

> But ingenious as these suggestions are, it must be admitted that they can hardly be treated as experimental hypotheses as long as the biochemical entities between which the interactions are imagined to take place remain vague or unspecified. Indeed as I have pointed out before, it is relevant that there never has been any direct biochemical evidence for the type of direct molecular interaction that Paul's conjectures would require between components of the respiratory chain (or photoredox chain) and ATPase systems. On the other hand there is good experimental evidence that the proton translocating respiratory chain and the proton translocating ATPase function independently…[56]

It is noteworthy that Mitchell treated Boyer's suggestions as those of the conformational theory and does not comment on Boyer's hybrid approach of a proton motive force that might drive the ATPase through a conformational mechanism.

Indeed it was the competing theories which tended to dominate discussion between the leading bioenergeticists at the time although Mitchell noted that Slater had recently abandoned the chemical theory – ''Bill recently wrote to us that the chemical hypothesis of the coupling mech-

[54] Letter, Boyer to Mitchell, 17 June 1974. P.D. Mitchell archive file G 77.

[55] Perutz, 1970. Although studies on the relationship between oxygen and haemoglobin had proceeded for half a century or more, it was this paper which had provided a structural basis for oxygen binding. For a more general account, see Perutz, 1998, pp. 255–277 and Ferry, 2007, pp. 223–230.

[56] Letter, Mitchell to Ernster, copied to Boyer, Chance, Racker, Slater and Snell, 12 August 1974 (P.D. Mitchell archive file G 77). It should be noted that the background to this letter and much of the correspondence at this time was disputes over the proposed collaborative review for the Annual Review of Biochemistry finally published in 1977, hence the wider circulation here. However the debate with Boyer on the ATP synthase began to be more public from this point on.

anism – of which he was formerly a most eloquent exponent – should now be considered untenable.''[57] Mitchell noted ''the biochemistry and physiology of the respiratory chain and the biochemistry of the ATPase… are profoundly coloured by the type of coupling mechanism that operates between them.''[58] The principal proposals for the coupling mechanism were now Mitchell's chemiosmotic hypothesis and Boyer's conformational hypothesis although Williams' views remained in contention.

Mitchell then wrote to Boyer to check his understanding of Boyer's views and included diagrams of his view of the conformational hypothesis, see Figure 2.[59] In his reply Boyer did not comment on Mitchell's diagrams but pointed out that

> The concept of conformational coupling is fundamentally distinct from other suggestions of how energy is used for ATP synthesis. It stresses that the conformational changes accompanying catalysis are not incidental but are the primary means by which energy is used to form the covalent bond in ATP.[60]

He then complicated matters by considering the conformational hypothesis, the chemiosmotic hypothesis and combinations of the two, all of which he was prepared to consider seriously. In another letter written on the same day, Boyer, an enzymologist, admitted again the difficulty in producing a mechanism for ATP synthesis involving a proton and indicated his specific objection to Mitchell's proposals on how proton or potential gradients might be used for synthesis. ''A molecular description of how ATP might be synthesized by a proton or potential gradient is a formidable problem that could take many years to solve.''[61]

In July 1974 Mitchell published a revised mechanism for the ATPase in which the proton passed directly through the enzyme and was involved in ATP synthesis at the active site but the unidentified intermediate of his 1966 proposal had disappeared. The paper included suggestions on how the proton participated in the catalytic mechanism.[62] Mitchell's paper was strongly criticised in a paper published by

[57] *Ibid*. See also Slater, 1974, p. 1153.

[58] Letter, Mitchell to Boyer, copied to Chance, Ernster, Racker Slater and Snell, 26 September 1974. P.D. Mitchell archive file G 77.

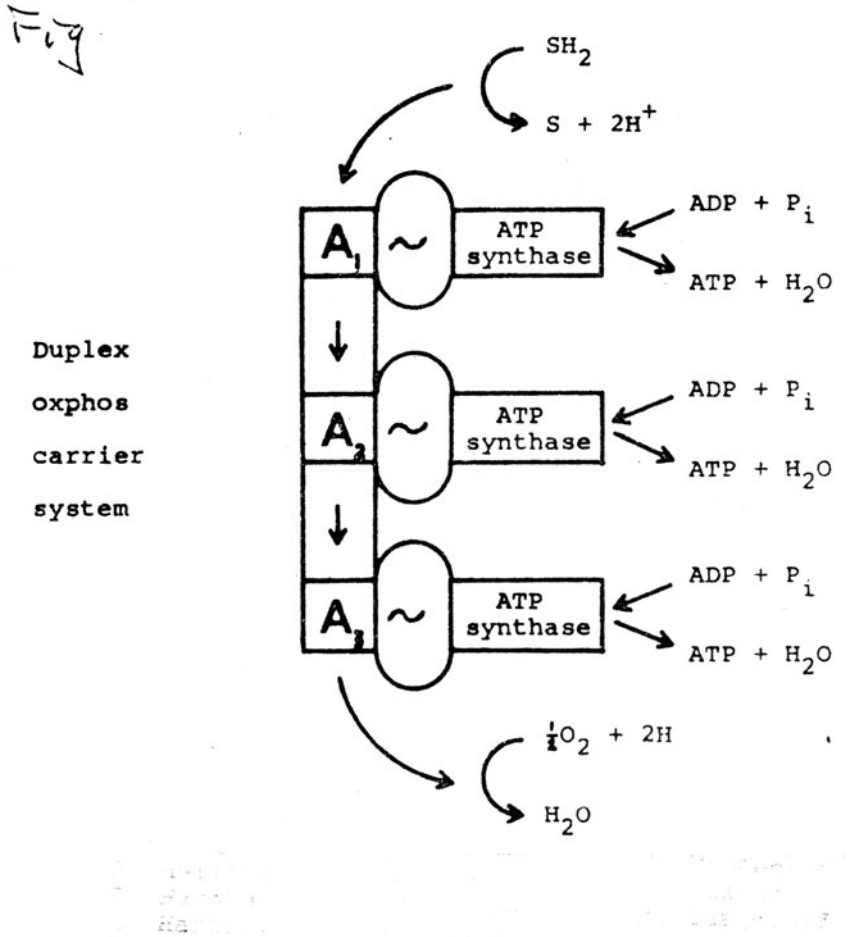
[59] Letter, Mitchell to Boyer, 17 October 1974. Note there are two letters of this date but this one was copied to 30 other workers in the field. P.D. Mitchell archive file G 77.

[60] Letter, Boyer to Mitchell, 21 November 1974; copied to Lumry, Azzi, Papa and Cross. Note there are two letters of this date. P.D. Mitchell archive file G 78.

[61] Letter, Boyer to Mitchell, 21 November 1974; copied to Ernster, Chance, Racker, Slater and Snell. P.D. Mitchell archive file G 77.

[62] Mitchell, 1974.

It is helpful, I think, to bring this idea to a diagrammatic form, as in the Figure given below.




Here $SH_2$ simply refers to hydrogenated substrates, and $A_1$ etc. refer to redox carriers. The bulgy part with the squiggle in represents (inevitably, rather crudely) the interacting polypeptides between the redox and ATP synthase reactions.

*Figure 2.* Mitchell's diagram for the conformational hypothesis. This was p. 2 of his letter of 17 October 1974 (copied to 30 other workers in the field). Mitchell commented in explanation of the diagram; "you suggest that coupling is achieved by transmission of energy through conformational interactions [~] between the proteins supposed to catalyse the redox reactions [$A_1$, $A_2$, $A_3$] on the one hand and the phosphorylation reactions on the other"

Boyer in the same journal the following February although a copy was sent to Mitchell in November 1974.[63] The criticisms were primarily of the proposed mechanism and made on chemical and thermodynamic grounds, particularly the involvement of an intermediate in the reaction ($O^{2-}$). The paper also now reiterated Boyer's suggestion on the possibility of a marriage between chemiosmotic and conformational ideas but he also noted that conformational coupling remained a possibility.[64] The fact that Boyer had published his views rather than discussed them in correspondence with Mitchell strained the relationship between the two. Mitchell wrote "I was sorry you did not, in answer to my invitation to help me out with my chemical ignorance, tell me personally about your criticisms of my ATPase paper before deciding to make them public."[65] Boyer replied that "I thought I had called your attention to what I felt were chemical limitations of your mode of using protons for ATP synthesis at the Bristol Symposium."[66] Boyer's published criticisms did change the tone of the correspondence for a while although other strains associated with the proposed cooperative review probably troubled Mitchell more. In these circumstances it should be noted that there is an underlying tone of very considerable respect that Boyer had for Mitchell – as Boyer wrote on one occasion: "I dislike being in apparent disagreement with one whose work I admire so much."[67]

Mitchell's revised hypothesis made little sense to Boyer, an accomplished chemist and enzymologist although Boyer seemed convinced of the possibility that an electrochemical proton gradient might be the means of conveying energy from the respiratory chain to the ATPase. Equally Boyer's conformational views were not acceptable to Mitchell who felt that they denied the central position of the vectorial proton essential to his view of the chemiosmotic hypothesis. This was sup-

[63] Boyer included a pre-publication copy of his paper in a letter copied to Ernster, Chance, Racker Slater and Snell. Boyer to Mitchell 21 November 1974. P.D. Mitchell archive file G 77.

[64] Boyer, 1975a. Mitchell was invited to respond to this paper by the editors of the journal, an action Boyer was unaware of and regarded as unethical (interview, Boyer 11 December 1993).

[65] Letter, Mitchell to Boyer, 10 January 1975. P.D. Mitchell archive file G 78. In addition to the comments in the correspondence, Mitchell formally replied to Boyer in February (Mitchell, 1975). Boyer found this response "unduly harsh." (Letter, Boyer to Mitchell, 4 April 1975 copied to 38 or 39 others). P.D. Mitchell archive file G 79).

[66] Letter, Boyer to Mitchell, 24 January 1975. P.D. Mitchell archive file G 78.

[67] Letter, Boyer to Mitchell, 8 December 1975 (copied to Chance, Ernster, Slater, Racker). P.D. Mitchell archive file G 80.

ported experimentally and in addition, he believed almost as an article of faith that the proton must be involved at the active site.

## Correspondence: Direct and Indirect Proton Coupling

For some time Mitchell seemed to ignore Boyer's suggestion in his letters and in his papers that a proton gradient might drive conformational changes leading to ATP synthesis. In a letter at the end of 1974, Boyer sought to persuade Mitchell to clarify his approach to conformational and chemiosmotic coupling.

> Your proton or potential gradients could cause conformational changes at parts of the phosphorylation complex spatially quite separated from the catalytic site. The change could be transmitted to the catalytic site by the interacting, 3-dimensional protein structure. In such instance, the conformational change provides the mechanism for use of the oxidative energy. Should such a hypothesis prove correct, would you not concur that conformational coupling had occurred?[68]

In reply Mitchell argued that the conformational changes to which Boyer had referred (essentially those within the ATP synthase) were a part of the notion of chemiosmotic coupling "which explicitly included the notion of conformational mobility."[69] This he believed he had described in a review in 1963 from which he quoted an extensive extract. The 1963 paper included a section on 'The Molecular Mechanism of Group- and Electron-translocation' that considered conformational changes as a part of the transfer of groups across biological membranes. This was part of a chemiosmotic process.[70] Mitchell distinguished the forgoing concept from Boyer's original conformational coupling concept which "was an important and novel concept because you introduced the idea that coupling between two chemical reactions, proceeding in two spatially separate catalytic centres, might be accomplished through the conformational movements of the intervening polypeptides."[71] It should be noted that by

[68] Letter, Boyer to Mitchell, 30 December 1974 (copied to Ernster, Chance, Racker, Slater, Snell and King). P.D. Mitchell archive file G 78.

[69] Letter, Mitchell to Boyer, 22 January 1975 (copied to 36 others). P.D. Mitchell archive file G 78.

[70] Mitchell, 1963, see p. 156.

[71] Letter, Mitchell to Boyer, 22 January 1975 (copied to 36 others). P.D. Mitchell archive file G 78.

this time the correspondence had really ceased to be private, the letter just referred to was circulated to 36 other workers in the field.

Boyer in reply felt that Mitchell was confusing the situation by extending his chemiosmotic idea to cover the conformational events.

> A hypothesis becomes meaningless or obscure if it attempts to encompass too much. In your recent FEBS Letters paper [ref.] you presented a mechanism for ATP synthesis based on the chemiosmotic hypothesis in which you suggested that energy from proton gradients was used for specific protonation of differing ionic forms of Pi at the catalytic site… I do not regard the suggestion as chemically attractive… The mechanism you suggest…is, on a molecular basis utterly distinct from the mechanism referred to… in which energy-linked conformational changes are used to change affinities or activities of rectants at the catalytic site. If any hypothesis becomes so broadly conceived or defined as to encompass two such distinct mechanisms for ATP synthesis, the hypothesis will cease to have value.[72]

The contrasting approaches of Boyer and Mitchell can be demonstrated by their reactions to a statement by Efraim Racker in which he suggested a marriage between the chemical, chemiosmotic and conformational hypotheses. This was regarded by Mitchell as "confusion masquerading as a gentleman's political compromise," a trap he was determined to avoid,[73] but positively by Boyer; "I find value in Ef's "compromise suggestions"; the possibility of energy transmission by proton gradient and use by conformational change looks like a way out of, not into a trap."[74]

Perhaps influenced by the recent correspondence with Mitchell on the possibility of combining the chemiosmotic and conformational approaches, Boyer published in October 1975 a proposal for a mechanism of oxidative phosphorylation which did combine both the chemiosmotic and conformational approaches. In essence a proton gradient created by the respiratory chain would bring about conformational changes in the ATPase leading to ATP synthesis; the mechanism being based on interactions between protons and charged groups on the protein

[72] Letter, Boyer to Mitchell, 19 February 1975 (copied to those listed in Mitchell's letter of 22 January). Underlining as in the original. P.D. Mitchell archive file G 78.

[73] Letter, Mitchell to Boyer, 22 January 1975 (copied to 36 others). P.D. Mitchell archive file G 78.

[74] Letter, Boyer to Mitchell, 19 February 1975 (copied to those listed in Mitchell's letter of 22 January). P.D. Mitchell archive file G 78.

surface. However, Boyer also noted that the direct conformational transfer of energy from respiration to the ATPase remained an attractive possibility. He felt that resistance to acceptance of Mitchell's proposals had arisen in part because of a lack of satisfactory suggestions on how the proton gradient and membrane potential could drive ATP synthesis.[75]

Following publication of his paper, Boyer sent a copy to Mitchell. In the accompanying letter he wrote that

> In retrospect, it took me far too long to recognize the importance of your work. Because I could not see a rational chemistry for the actual synthesis of ATP, I was reluctant to give attention to what I feel is the crucial aspect of your chemiosmotic hypothesis, and what I think most scientists think of when they refer to the chemiosmotic hypothesis. This is that oxidations can give rise to a potential or proton gradient across a membrane, and that such gradients can be used to make ATP. How oxidations may cause proton translocation, or how protons may be used to make ATP is not what most people think of when 'chemiosmotic' is mentioned.[76]

Referring also to Boyer's paper (Boyer, 1975b, included in the letter of 7 August 1975[77]), Mitchell replied:

> I could find nothing whatever about the ''chemistry for the actual synthesis of ATP'' but only general statements to the effect that the phosphorylation of ADP is accomplished by ''conformational coupling''. You do not like my (amateurish) attempts to indicate the general type of chemical mechanism in which the $H^+$ ions and the other components of the ATPase reaction might interact. Yet you put nothing in its place. I wish I could encourage you to write better direct chemiosmotic mechanisms instead of only criticizing destructively.[78]

Mitchell went on to note that Boyer's conformational proposals required the exclusion of the proton from the components of the ATPase reaction.

In the summer of 1975, following several letters largely concerned with terminology, Boyer began to feel that little real progress was being

[75] Boyer, 1975b.

[76] Letter, Boyer to Mitchell, 8 December 1975 (copied to Chance, Slater, Ernster, Racker). P.D. Mitchell archive file G 80.

[77] P.D. Mitchell archive file G 80.

[78] Letter, Mitchell to Boyer, 29 December 1975 (copied to Chance, Slater, Ernster, Racker). P.D. Mitchell archive file G 80.

made with the correspondence and suggested its termination, "I feel I should withdraw from my participation in the correspondence."[79] In a later letter he noted, "Even though I do not feel it useful to continue with a semi-public dialogue, I would welcome continuing personal correspondence."[80] In fact the correspondence discussing the ATPase (and other issues not considered here) continued to the end of 1975 when it terminated, although it was resumed later.[81] However among those other issues was Mitchell's concern that Boyer and others failed to distinguish between the proton or pH gradient and the proton electrochemical potential together with much debate about terminology, an issue close to Mitchell's heart.

Mitchell's position had not really changed and was well summed up by Slater. "Peter Mitchell believes, however, that protons transmitted through a hole to the active site of the enzyme play a direct role in the phosphorylation reactions."[82]

## Review

In assessing this discussion between Boyer and Mitchell, there are background matters to consider. The correspondence on the enzyme is embedded in a wider debate about publishing a joint review. This was initiated by Efraim Racker in 1974 on the grounds that the field was in such turmoil that funding agencies in the U.S. were beginning to lose interest in supporting bioenergetics. In response, Boyer, then Associate Editor of the *Annual Review of Biochemistry* proposed a review of the field to be published in that journal, copying his letter to a number of other leading bioenergeticists together with the Editor of the *Annual Review*, Esmond Snell (1914–2003). There then followed a protracted wrangle about the nature of such a review.[83]

Several of the letters quoted cover both the review and the enzyme although on a few occasions two letters were written on the same day, one on each topic.[84] In general Mitchell was defending his position.

[79] Letter, Boyer to Mitchell, 9 June 1975 (copied to 38 others). P.D. Mitchell archive file G 79.

[80] Letter, Boyer to Mitchell, 7 August 1975. P.D. Mitchell archive G 80.

[81] There were occasional letters between 1976 and 1979 but discussion of scientific issues did not resume until 1984.

[82] Slater, 1976, see p. 50.

[83] Weber and I have discussed this in Prebble and Weber, 2003, pp. 195–208.

[84] For example, letters of Mitchell on 17 October 1974 and of Boyer on 21 November 1974. P.D. Mitchell archive file G 77.

While his discussion of the review was usually copied to those he and also Boyer recognized as leaders in the field (Chance, Ernster. Racker, and Slater and also Snell, the editor), the discussion of the enzyme was circulated more widely. In Mitchell's two letters of 17 October 1974, the review one was copied to the usual group but the enzyme letter to 30 others. The membership of the latter group varied but reached a total of 41 on May 7 1975. It is clear here that Mitchell is appealing to the wider field of bioenergeticists to understand his position. Boyer normally copied his letters to those previously selected by Mitchell. The circulation of the correspondence ceased after 1975 at Boyer's request and the later debate of the 1980s, which was private, follows a period when Mitchell, now a Nobel laureate no longer needed to persuade the field.

The wrangling over the review had opposite effects on Boyer and Mitchell. As Mitchell commented, he felt himself to be "in a minority of one"[85] and was defensive, a position expressed in terms of his circulation of his arguments to the wider field. Boyer was seeking a basis for common ground and became willing to marry his views with those of Mitchell.

Finally a review of oxidative phosphorylation and photophosphorylation composed of six separate essays each by a different author, including both Mitchell and Boyer, was published in 1977 together with an agreed page of introduction.[86] Mitchell's participation was in doubt until very late in the preparation of the manuscript. While not abandoning the conformational theory totally, Boyer now advanced the notion of coupling through an electrochemical proton gradient but more especially a conformational process for ATP synthesis by the synthase. Others such as Slater also considered the conformational hypothesis as a possibility. Boyer summarised his view of the energetics of ATP synthesis as follows.

> A satisfying explanation of how ATP is made must include a mechanism for synthesis coupled to the use of a transmembrane potential or proton gradient. This poses at least as difficult a problem as earlier suggestions of more direct coupling to oxidations for which plausible chemical and biochemical models can be suggested. Although energy use through conformational change provides a rational explanation for present data, proof in molecular terms for the conformational events, if obtainable, is some years away.[87]

[85] The comment was made in relation to the proposed review where Mitchell felt isolated against his colleagues whose sympathy for the chemiosmotic hypothesis was limited. Letter Mitchell to Ernster, copied to 5 others. P.D. Mitchell archive file G 77.

[86] Boyer et al., 1977.

[87] Boyer et al., 1977, see p. 961.

In this review, Boyer also concluded with a description of his alternating site hypothesis, a development of his view of the conformational process in the ATP synthase involving two, rather than one, catalytic sites on the enzyme. Both Slater and Boyer stressed the fact that the energy for the ATP synthase is used for ATP release from the enzyme which is usually taken to imply conformational change of the enzyme.

In the same review, Mitchell also regarded the details of the mechanism in the ATPase as 'conjecture' but expressed his opinion that protons are involved at the active site. In Mitchell's 1978 Nobel lecture,[88] he retained his view that the proton was involved in the active site of the ATPase. He reiterated his belief that "It was my opinion that the biochemical content and value of the chemiosmotic rationale depended on the feasibility of proton motive chemiosmotic reaction mechanisms of the direct group-translocation type," such as the direct involvement of the proton in the active site of the ATPase. Thus the gap between Mitchell, who favoured direct proton involvement in ATP synthesis and Boyer and most others who favoured indirect proton involvement was widening.

## Revised Hypotheses for the ATP Synthase

The rapidly developing understanding of the ATP synthase in the 1980s included a detailed subunit structure for the enzyme, particularly the nine subunits of the catalytic portion, $F_1$, in the bacterial enzyme. Although there had been much uncertainty about the actual subunit number, Boyer was impressed by the work of Kagawa's group in Japan who concluded that there were three copies of the catalytic subunit, beta, where ATP synthesis took place.[89] Concurrently, the experimental work in Boyer's laboratory, as noted above, had already led to his alternating site view of the enzyme catalysis which explained the way two sites worked not independently, but co-operatively in the enzyme. Five years later Boyer was driven to the conclusion that there were three catalytic subunits, as Kagawa's group had shown, working co-operatively.[90] The only way he could explain this was in terms of a rotational model in which part of the enzyme (the gamma subunit) rotated.

[88] Mitchell, 1979.

[89] Kagawa et al., 1976.

[90] The alternating model for the enzyme was described in Kayalar et al., 1977 but the rotational model was formally communicated in a paper, Gresser et al. in 1982, although discussed at Gordon conferences before that.

This rotation of a central subunit led to conformational changes in the catalytic beta-subunits driving ATP synthesis.[91]

The new knowledge posed significant challenges for Mitchell who remained convinced that the proton should be involved in the catalytic site. Accordingly he proposed new mechanisms in 1981 (where he claimed to have considered rotatory catalysis before Boyer!) and particularly in 1985 where rotating catalytic subunits were involved.[92] In the latter, the central subunit of the enzyme, known as gamma, acted as a rotating proton gate so that the protons reached each catalytic site in turn. Mitchell felt he had strong reasons for proton involvement in the ATP synthase and this confidence had been strengthened by the Nobel Prize. However, his mechanistic proposals lacked experimental support and paid only scant attention to the experimental evidence being obtained by Boyer, Slater and several other groups.

Boyer had visited England in the early summer of 1984 and briefly stayed at Glynn, Mitchell's private research institute and also his home. The discussions at Glynn were continued in a renewed correspondence. Although the differences with Boyer were still there, they were now in some ways closer together; as Mitchell noted "I remember Lasse Ernsters's public comment about my ternary state mechanism (written for his Festschrift) at the Gordon Conference in 1981 that it looked as if our ATPase models were quite similar."[93] Mitchell asked Boyer "why you have concluded that your work is inconsistent with the direct type of chemiosmotic coupling mechanism discussed in some of my papers?" Mitchell went as far as suggesting that he and Boyer should "make a collaborative effort to build a mechanistic scheme that seems satisfactory to both of us?"[94]

Boyer's reply was lengthy and started with his acceptance of what he regarded as the core of the chemiosmotic concept, namely that the proton motive force can drive ATP synthesis. He noted that some aspects of Mitchell's schemes were "appealing" and considered areas where the

[91] Boyer first put forward his rotational catalysis model in the early 1980s, see Gresser et al., 1982. It should be noted that I have only considered here ATP synthesis. However it was essential that any mechanism for the enzyme was reversible explaining ATP breakdown as well.

[92] Mitchell, 1981 produced a mechanism based on three catalytic sites and retaining a role for the proton at each of them. Mitchell, 1985 proposed a complex rotation of each of the seven main subunits but again the proton was active at the three catalytic sites.

[93] Letter, Mitchell to Boyer, 4 July 1984. P.D. Mitchell archive file B134.

[94] Letter, Mitchell to Boyer, 9 June 1984. This and two other letters of this period are not in the Mitchell archive; I am grateful to Professor Boyer for copies.

two were more or less in agreement. The principle disagreement appeared where Mitchell required proton energy for a transition step in the enzyme mechanism for which Boyer now had evidence that energy was not required. Boyer also noted his objection to Mitchell's hypothetical intermediate, as he had done previously that "the participation of an $O^{2-}$ group is chemically unattractive." Later in the letter he noted that

> It is conceivable that movement of the translocated protons through $F_0$ and part of the $F_1$ occurs… However, it seems much more likely, on the basis of accumulating wealth of observations of properties of proteins that changes in conformation caused by proton translocation are transmitted indirectly through the protein matrix to the catalytic sites.

He went on to note the relevance of work on lactose transport in bacteria and commented that "Indirect conformational coupling provides a very appealing general way to use Mitchellian proton motive force."[95]

Despite the fact that the main progress in the field was being made by Boyer and others who shared his views, Mitchell retained his belief that protons must be involved at the active site. In what was probably the last letter from Boyer to Mitchell, he wrote rather directly that "I feel the present data definitely favour the indirect coupling mechanism [in] which the translocated proton does not reach the catalytic site area. Thus although I like the sequential participation of your cyclic ternary state, I have little enthusiasm for the suggested mode of ATP formation."[96] Mitchell was now totally out of step with those at the cutting edge of the field. This was reinforced by a paper he gave at the silver jubilee meeting held to celebrate 25 years of his research institute, the Glynn Research Foundation. The newer approach to structure was included but the mechanism was still the direct involvement of protons.[97] This was one of his last contributions to the debate; he died on 10 April 1992. Boyer's view was given strong support from the extensive structural studies of the enzyme by Walker's group leading in due course to a Nobel prize shared with Walker.[98]

[95] Letter, Boyer to Mitchell, 28 July 1984. P.D. Mitchell archive file G 81. Work on lactose transport in the bacterium, *Escherichia coli* (together with work on the regulation of amino acid synthesis), had led Monod's group to an understanding of the importance of conformational changes in proteins (see Monod et al., 1963).

[96] Letter, Boyer to Mitchell, 26 February 1987. P.D. Mitchell archive file G 81.

[97] Mitchell, 1991.

[98] Abrahams et al., 1994. The 1997 Nobel prize was given half for the ATP synthase work and half for the $(Na^+\text{-}K^+)$-ATPase. The latter half was awarded to Jens Skou and the former was shared by Boyer and John Walker.

## Styles of Biochemistry

The forgoing correspondence reveals a significant contrast in styles of Boyer and Mitchell in their approach to biochemistry. There have been various considerations of 'style,' for example by Fruton comparing primarily research group leadership by Fischer and Hofmeister, by Harwood who provided definitions of 'style' when exploring styles of scientific thinking in German geneticists and by Kohler documenting the institutional development of biochemistry.[99] Here the issue has its own unique characteristics.

It is a curiosity that the man who transformed the understanding of oxidative phosphorylation in the 1960s and laid the basis for the understanding of the ATP synthase should have so signally failed to grasp the process by which energy would be available for ATP synthesis itself. It had proved difficult for Mitchell to persuade the field of the importance of a proton gradient and membrane potential in acting as the intermediary between oxidation and phosphorylation but eventually his colleagues were convinced. Once that had been achieved it followed that the mitochondrial, chloroplast and the enteric bacterial ATP synthases were proton-translocating enzymes. This was Mitchell's totally novel contribution to biochemistry. But as mechanisms of the enzyme itself began to be appreciated, particularly in Boyer's laboratory, Mitchell clung to a belief for which he had little evidence, that the proton must be involved at the active site of the enzyme where ATP was formed from ADP and phosphate. He seemed incapable of coming to terms with the indirect conformational mechanism progressively developed by Boyer and others. This contrasts sharply with Boyer who in the 1970s progressively incorporated the proton gradient and membrane potential into his conception of oxidative phosphorylation. Why should so gifted a scientist behave in such an obstinate way?

The contrasting backgrounds of Boyer and Mitchell are an important element in understanding the differences in style and their approaches to biochemistry. Boyer took a first degree in chemistry at Brigham Young University and then doctoral studies in biochemistry at the University of Wisconsin where he was encouraged to study the developing field of enzymology. Here he began an interest in bioenergetics stimulated in part by the notable "Symposium on Respiratory Enzymes" held at the University of Wisconsin in 1941 where he listened to several of the leaders in contemporary biochemistry

[99] Fruton, 1985; Harwood, 1993; Kohler, 1982.

including Otto Meyerhof on intermediate metabolism, Herman Kalckar on phosphorylation and Fritz Lipmann on the Pasteur effect.[100] The outcome of his doctoral work was the identification of an essential monovalent cation, $K^+$, in the pyruvate kinase reaction.[101]

After a spell in Stanford, he moved to the University of Minnesota where, among other things, in the mid-1950s he carried out experiments on ATP synthesis using an oxygen isotope (briefly discussed earlier); these studies laid the basis for much of his later work on the ATP synthase. In June 1963 he joined the Chemistry Department of UCLA where work on oxidative phosphorylation continued. In 1965, Boyer became the Director of the new Molecular Biology Institute which, under his leadership took shape with its own building opening in 1977.[102] Two elements of this story are especially relevant to Boyer's approach to bioenergetics. Firstly, his strong chemical background which is also evidenced in his letters. Secondly, the association with enzymology from his early doctoral work through to work on ATP synthesis in mitochondria. His approach to bioenergetics and his correspondence is that of an enzymologist. Thus his style of biochemical investigation is that of a chemist and enzymologist.

This background contrasts with that of Mitchell. He was a student in the Cambridge (England) Biochemistry Department in the last days of the leadership of Frederick Gowland Hopkins who developed a general Biochemistry Department unfettered by medical requirements but with a broad biological outlook.[103] Influential on Mitchell in his Cambridge days (1939–1955) were those with a strong biological bias, Danielli who worked on membranes, Gale, a microbiologist and Keilin at the Molteno Institute who worked on the respiratory chain (but not the associated phosphorylation except in conjunction with Slater).[104]

Another influential element of this period was his interest in biological philosophy, particularly that of Joseph Woodger (1894–1981) who wrote *Biological Principles* (1919).

[100] University of Wisconsin, 1942.

[101] This is the second ATP synthesizing enzyme in glycolysis.

[102] Boyer, 1981, 1998a.

[103] Kohler, 1982 describes the Cambridge Department as "a daring realization of Hopkin's vision of general biochemistry" (see p. 84) in distinction to many North American departments which, during the earlier part of the twentieth century, were mostly developed under the guidance of medical faculties, or less frequently influenced by chemical and only in a few cases by biological forces.

[104] See Prebble and Weber, 2003, where Mitchell's Cambridge period is described, pp. 24–63.

Hopkins had been concerned with the biochemistry of small molecules and this view influenced the development of his department.[105] Mitchell remembered the lectures of the enzymologist, Malcolm Dixon, where the enzyme itself was drawn on the board as a box and its reactants shown in relation to the box, an approach reflected in Mitchell's diagram in Figure 2.[106] From an examination of his published papers, it can be seen that Mitchell's biochemical interest was in small molecules and ions.

Thus Mitchell's style of biochemistry, conditioned by the Cambridge Department and his strong macro-biological philosophy[107] and worked out in terms of small metabolites and ions contrasts dramatically with Boyer's molecular biology of enzymes as proteins emerging from a strong background in chemistry. This goes some way to explaining the marked difference in the significance attached to the chemistry of proposed reactions. Mitchell, a physiological biochemist, employed chemistry which was heavily criticized by Boyer, a trained chemist and also by another distinguished chemist, Bob Williams, who commented "that Mitchell had no mechanism in any molecular sense for ox. or photo phos (which is very peculiar)."[108] The difference is epitomised by Boyer's early comments on phosphorylations: "From a chemist's viewpoint, a primary requisite for understanding the mechanism of a reaction sequence is the description of all the covalent bonds that are formed and broken in the process."[109] and Mitchell's wish to define chemical entities expressed in his August 1974 letter, referred to earlier.

The contrast in styles of biochemistry was deeper than just that derived from their university training and disciplinary links. Mitchell was at heart a theoretician and his proposals, particularly those relating to the ATP synthase were derived from his speculative consideration of

[105] In an early lecture, Hopkins (1913) emphasised the significance of biochemistry as 'dynamic' and strongly stressed the importance of small molecules: "In the study of the intermediate processes of metabolism we have to deal, not with complex substances which elude ordinary chemical methods but with simple substances undergoing comprehensible reactions". As Kamminga points out, Hopkins began to put his vision into practice during the 1920s and established the ethos of the department (Kamminga, 2002). The situation began to change when Chibnall became head of department in 1943 and introduced a privileged line of research into plant proteins (García-Sancho, 2010) but the influence of Hopkins remained.

[106] Interview with Mitchell 15 March 1991.

[107] See Prebble, 2001.

[108] Letter, Williams to Boyer, 10 July 1975. I am grateful to Professor Boyer for a copy of this letter.

[109] Boyer, 1965, p. 994.

membrane-bound enzymes. On the back of the theory, many speculative mechanisms were proposed. As the chairman of the committee which recommended Mitchell for the Nobel Prize has noted, "Mitchell was right only on the phenomenological and not on the mechanistic level."[110] Although this was not entirely true since as I have pointed out elsewhere, the essence of his Q cycle mechanism has stood the test of time,[111] it nevertheless summarises much of Mitchell's contribution to biochemistry. Nowhere was this aspect of Mitchell's work more in evidence than in his approach to the key enzyme of oxidative phosphorylation, the ATP synthase.

By contrast, after an initial speculative approach in the mid-1960s, Boyer built his ideas on conformational changes in the ATP synthase as a result of seeking interpretations of his, often puzzling, experimental results. According to his autobiographical notes, his initial formulation of the conformational approach to the ATP synthase itself was a consequence of his endeavour to understand his experimental results.

Another difference in the style of the two scientists lies in their institutional background. Boyer had built the Molecular Biology Institute at Los Angeles and had been an integral part of his University. At the end of his Nobel lecture, he expressed his indebtedness to "the universities and government agencies that provided the environment and the financial support for my researches."[112] He was very much a team player being part of the group that edited the *Annual Reviews* as well as many other activities.

Mitchell was much more of a loner. After moving from Cambridge to Edinburgh in 1956 where he formulated his hypothesis, he became ill and had to leave the University in the early 1960s. Having substantial family resources and together with his associate scientist, Jennifer Moyle, he set up his own institute at Bodmin, Cornwall, in the relatively remote South-west of England. Here he set out to test both his hypothesis and whether it was still possible in the second half of the twentieth century to successfully build a small private research institute. Most of the work he funded himself and external grants were limited. In contrast with Boyer who had a long run of able co-workers, Mitchell had few. Mitchell's isolation at his institute was enhanced by a disastrous operation on his ear in 1972 which left him seriously deaf for the

[110] Malmström, 2000.

[111] The Q cycle proposed in 1974 was a mechanism for proton translocation across the mitochondrial membrane by a section of the respiratory chain in mitochondria (Prebble, 2000).

[112] Boyer, 1998b.

rest of his life. In consequence he found scientific meetings very difficult and tended to avoid them. Mitchell's remoteness to the rest of the field was noted by Britton Chance during the long debate seeking agreement for the 1977 review, "Perhaps one of our problems is the isolation of Bodmin. It is hard to get to and Peter has been unable to travel."[113] A consequence of these problems was a voluminous correspondence. His science reflected this independence, sometimes absolute brilliance, but occasionally almost quixotic but almost always original and frequently unorthodox.[114] Hence his major contribution has been seen by another scientist as "counterintuitive."[115]

Thus Mitchell and Boyer differed both in their institutional and disciplinary backgrounds accounting at least in part for their contrasting approaches to the problems of bioenergetics.

## The Molecular Biological Influence

An important step in the history of bioenergetics is illustrated in the Boyer-Mitchell correspondence. This is the impact of protein technology on the experimentation and understanding of the machinery of oxidative phosphorylation. Because the system is membrane-bound it was not open to the earlier techniques for protein study. The exception was cytochrome *c* which is easily detached from the inner mitochondrial membrane and can readily be purified in its native form. While the knowledge and accessibility of cytochrome *c* substantially aided the study of oxidative phosphorylation, the relative lack of accessibility of the major components was an important factor in limiting progress in the 1960s and 1970s. As noted above, workers in bioenergetics concentrated on the events concerning small molecules, leaving the proteins themselves as 'black boxes'. The difficulty in applying protein chemistry can be seen in Boyer's distrust of the results of much work on the number of subunits in the ATP synthase which had not given consistent results, and his assessment of the work of Kagawa's group as probably indicating the true stoichiometry. Boyer was part of the new approach to bioenergetics which recognized that understanding the mechanism of oxidative phosphorylation would require an understanding of the

[113] Letter, Chance to Slater, 25 April 1974; courtesy of the late Professor Britton Chance.

[114] Weber and I have explored some aspects of this in our contribution to "Rebels, Mavericks and Heretics in Biology" (Prebble and Weber, 2008).

[115] Orgel, 2000.

proteins themselves.

As Ernster and Schatz remarked when writing a history of the mitochondrion in 1981, progress in understanding oxidative phosphorylation was passing "from the membranologist to the protein chemist."[116] The study of systems which remained bound to membranes, so expertly promulgated by Mitchell and many others, had been successful but further progress would now depend on the investigation of the proteins, their structures and their interactions. This had not really been a significant part of Mitchell's scientific view and he found himself unable to empathize with it, possibly because of his isolation. Although an avid reader of the literature, he did not relate to others at conferences and in a day-to-day university or research Institute[117] setting whereas Boyer, an associate editor of the leading review journal in biochemistry, was a social part of the leading edge of his subject. It was in enzymology, Boyer's own field where the ideas of protein conformation had been very successfully developed with the notion of allosteric control of enzyme reactions by Wyman and by Monod's group.[118]

For a long time Boyer did not appreciate the significance of the notion that the energy of respiration could be made available in proton gradients since he could not see how the proton could drive ATP synthesis. However, after about 1974 by which time the chemiosmotic hypothesis was accumulating significant experimental support, he recognised the way such a system might function together with the conformational processes he was describing for the enzyme. Mitchell who was rather less concerned about his chemistry placed much emphasis on the stoichiometry of ATP synthesis. For most of this period Mitchell adhered to the stoichiometry of three ATPs synthesised per oxygen atom reduced in respiration. He strongly felt that conformational mechanisms would not support a fixed stoichiometry. He seemed incapable of recognizing the potentialities of energy associated with conformational changes. In part this is well illustrated in a letter he wrote to the enzymologist, William Jencks (1927–2007).

> The notion of enzyme catalyzed group translocation, which was the main point of departure for the development of ideas about

[115] Orgel, 2000.

[116] Ernster and Schatz, 1981, see p. 247s.

> chemiosmotic reactions, has always contained an essential attribute of strictly articulated conformational change in which the enzyme complex participates as a whole… As Vladimir Skulachev once said, one can explain biochemistry on the basis that "proteins can do anything" – hence the magical significance that has tended to be attached to "conformational" notions. I suppose I have got myself into trouble by trying to think of the vectorial diffusional processes required in chemiosmotic reactions directly in terms of specific mechanisms involving reactive groups – as in classical enzymology – rather than invoking the black-box type of conformational idea where the coupling mechanism can be left for the protein to take care of by means of its power "to do anything".[119]

In fact, Mitchell himself regarded his most important contribution to science, not as the chemiosmotic theory but as his understanding of the vectorial (directional in space) nature of living processes. He felt that thinking in biochemistry and related science was in scalar terms whereas in reality living things had a strong vectorial element to their reactions. The chemiosmotic theory where protons reacted directionally across membranes became just an example of this basic understanding. To Mitchell conformational processes might be a necessary part of vectorial processes but they did not seem to be inherently vectorial. To satisfy his vectorial approach, the proton needed to be involved in the ATP synthesis itself.

There were perhaps more general reasons for a dispute between Mitchell and Boyer. Mitchell was given to being stubborn and unwilling to concede that others might have the right approach. Such was the case in the long argument over whether cytochrome oxidase pumped protons where Mitchell held out for several years before finally being convinced in 1985. A parallel argument proceeded over proton stoichiometry.[120] Thus the debate over the mechanism of the ATP synthase was not unique in its overall character. However, unlike the other debates, the issue of the ATPase was never resolved. Indeed when Mitchell was having difficulty persuading his colleagues of his own theory, he occasionally referred to a saying attributed to Max Planck: "A new scientific

[119] Letter, Mitchell to Jencks, 26 May 1977 (P.D. Mitchell archive file G 471). Vladimir P. Skulchev (1935–), Moscow State University, was the leading Russian bioenergeticist of the period.

[120] See Prebble, 2002. The story of the arguments over stoichiometry and cytochrome oxidase proton pumping is also explored in Chapters 9 and 11 of Prebble and Weber (2003). Mitchell's unorthodox approach to science is explored in Prebble and Weber (2008).

truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die, and a new generation grows up that is familiar with it."[121] Sadly, so far as the indirect role of protons in the ATPase is concerned, such a quotation became relevant to Mitchell. However, perhaps the key to understanding the correspondence between Mitchell and Boyer is to be found in the fact that the bioenergetics of the 1980s was now increasingly using the technologies provided by molecular biology whereas Mitchell was grounded in pre-1980 biochemistry.

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## References

Abrahams, Jan Pieter, Leslie, Andrew G.W., Lutter, René, and Walker, John E. 1994. "Structure at 2.8 Å Resolution of $F_1$-ATPase from Bovine Heart Mitochondria." *Nature* 370: 621–628.

Allchin, Douglas. 1997. "A Twentieth Century Phlogiston: Constructing Error and Differentiating Domains." *Perspectives in Science* 5: 81–127.

——2002. "To Err and Win a Nobel Prize: Paul Boyer, ATP Synthase and the Emergence of Bioenergetics." *Journal of the History of Biology* 35: 149–172.

Bechtel, William, and Abrahamsen, Adele. 2007. "In Search of Mitochondrial Mechanisms: Interfield Excursions Between Cell Biology and Biochemistry." *Journal of the History of Biology* 40: 1–33.

Boyer, Paul D. 1965. "Carboxyl Activation as a Possible Common Reaction in Substrate-Level and Oxidative Phosphorylation and in Muscle Contraction." Tsoo E.

King, H.S. Mason, and M. Morrison (eds.), *Oxidases and Related Redox Systems*, vol. 2. New York: Wiley, pp. 994–1008.

——1968. "Oxidative Phosphorylation." Thomas P. Singer (ed.), *Biological Oxidations*. New York: Interscience, pp. 193–235.

——1975a. "Energy Transduction and Proton Translocation by Adenosine Triphosphatases." *FEBS Letters* 50: 91–94.

——1975b. "A Model for Conformational Coupling of Membrane Potential and Proton Translocation to ATP Synthesis and to Active Transport." *FEBS Letters* 58: 1–6.

——1981. "An Autobiographical Sketch Related to My Efforts to Understand Oxidative Phosphorylation." Giorgio Semenza (ed.), *Of Oxygen Fuels and Living Matter Part 1*. Chichester: John Wiley and Sons Ltd, pp. 229–264.

——1998a. "Paul D. Boyer." *Les Prix Nobel 1977*. Stockholm: The Nobel Foundation, pp. 177–185.

——1998b. "Energy, Life and ATP." *Les Prix Nobel 1977*. Stockholm: The Nobel Foundation, pp. 186–207.

Boyer, Paul D., Bieber, Loran L., Mitchell, Robert A., and Szabolcsi, G. 1966. "The Apparent Independence of the Phosphorylation and Water Formation Reactions from the Oxidation Reactions of Oxidative Phosphorylation." *Journal of Biological Chemistry* 241: 5384–5390.

Boyer, Paul D., Chance, Britton, Ernster, Lars, Mitchell, Peter, Racker, Efraim, and Slater, Edward C. 1977. "Oxidative Phosphorylation and Photophosphorylation." *Annual Review of Biochemistry* 46: 955–1026.

Boyer, Paul D., Cross, Richard L., and Momsen, William. 1973. "A New Concept for Energy Coupling in Oxidative Phosphorylation Based on a Molecular Explanation of the Oxygen Exchange Reactions." *Proceedings of the National Academy of Science USA* 70: 2837–2839.

Boyer, Paul D., Falcone, A.B., and Harrison, W.H. 1954. "Reversal and Mechanism of Oxidative Phosphorylation." *Nature* 174: 401–402.

Chance, Britton, and Williams, G.R. 1956. "The Respiratory Chain and Oxidative Phosphorylation." *Advances in Enzymology* 17: 65–134.

Cohn, Mildred. 1953. "A Study of Oxidative Phosphorylation with $O^{18}$-Labelled Inorganic Phosphate." *Journal of Biological Chemistry* 201: 735–750.

Ernster, Lars, and Schatz, Gottfried. 1981. "Mitochondria: A Historical Review." *Journal of Cell Biology* 91: 227s–255s.

Fernández-Morán, Humberto. 1962. "Cell-Membrane Ultrastructure. Low Temperature Electron Microscopy and X-Ray Diffraction Studies of Lipoprotein Components in Lamellar Systems." *Circulation* 26: 1039–1065.

Fernandez-Morán, Humberto, Oda, T., Blair, P.V., and Green, David E. 1964. "A Macromolecular Repeating Unit of Mitochondrial Structure and Function." *Journal of Cell Biology* 22: 63–100.

Ferry, Georgina. 2007. *Max Perutz and the Secret of Life*. London: Chatto & Windus.

Fruton, Joseph S. 1985. "Contrasts in Scientific Style. Emil Fischer and Franz Hofmeister: Their Research Groups and Their Theory of Protein Structure." *Proceedings of the American Philosophical Society* 129: 313–370.

——1999. *Proteins, Enzymes, Genes*. New Haven: Yale University Press.

García-Sancho, Miguel. 2010. "A New Insight into Sanger's Development of Sequencing: From Proteins to DNA, 1943–1977." *Journal of the History of Biology* 43: 265–323.

Green, David, and Baum, Harold. 1970. *Energy and the Mitochondrion*. New York: Academic Press.

Gresser, Michael J., Myers, Jill A., and Boyer, Paul D. 1982. "Catalytic Site Cooperativity of Beef Heart Mitochondrial $F_1$ Adenosine Triphosphatase." *Journal of Biological Chemistry* 257: 12030–12038.

Harwood, Jonathan. 1993. *Styles of Scientific Thought. The German Genetics Community 1900–1933*. Chicago: University of Chicago Press.

Holmes, Frederic L. 1991. *Hans Krebs. The Formation of a Scientific Life 1900–1933*, vol. 1. New York: Oxford University Press.

——1992. *Between Biology and Medicine: The Formation of Intermediary Metabolism*. Berkeley: University of California.

Hopkins, Frederick Gowland. 1913. *The Dynamic Side of Biochemistry*. Reprinted in Joseph Needham and Ernest Baldwin (eds.), *Hopkins and Biochemistry 1861–1947*. Cambridge: Cambridge University Press.

Jagendorf, André, and Uribe, Ernest. 1966. "ATP Formation Caused by Acid–Base Transition of Spinach Chloroplasts." *Proceedings of the National Academy of Science USA* 55: 170–177.

Kagawa, Yasuo, and Racker, Efraim. 1966. "Partial Resolution of the Enzymes Catalysing Oxidative Phosphorylation. X. Correlation of Morphology and Function in Submitochondrial Particles." *Journal of Biological Chemistry* 241: 2475–2482.

Kalckar, Herman M. 1941. "The Nature of Energetic Coupling in Biological Syntheses." *Chemical Reviews* 28: 71–178.

Kamminga, Harmke. 2002. "Hopkins and Biochemistry." Peter Harman and Simon Mitton (eds.), *Cambridge Scientific Minds*. Cambridge: Cambridge University Press, pp. 172–186.

Kayalar, Celik, Rosing, Jan, and Boyer, Paul D. 1977. "An Alternating Site Sequence for Oxidative Phosphorylation Suggested by Measurement of Substrate Binding Patterns and Exchange Reaction Inhibitions." *Journal of Biological Chemistry* 252: 2486–2491.

Kohler, Robert E. 1982. *From Medical Biochemistry to Biochemistry. The Making of a Biomedical Discipline*. Cambridge: Cambridge University Press.

Lipmann, Fritz. 1946. "Metabolic Process Patterns." *Currents in Biochemical Research*. New York: Interscience Publishers, pp. 137–148.

Malmström, Bo. 2000. "Mitchell Saw the New Vista, If Not the Details." *Nature* 403: 356.

Mitchell, Peter. 1961. "Coupling of Phosphorylation to Electron and Hydrogen Transfer by a Chemi-Osmotic Type of Mechanism." *Nature* 191: 144–148.

——1963. "Molecule, Group and Electron Translocation Through Natural Membranes." *Biochemical Society Symposium* 22: 142–169.

——1966a. "Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation." *Biological Reviews* 41: 445–502.

——1966b. *Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation*. Bodmin, England: Glynn Research Ltd.

——1974. "A Chemiosmotic Molecular Mechanism for Proton-Translocating Adenosine Triphosphatases." *FEBS Letters* 43: 189–194.

——1975. "Proton Translocation Mechanisms and Energy Transduction by Adenosine Triphosphatases: An Answer to Criticisms." *FEBS Letters* 50: 95–97.

——1979. "Keilin's Respiratory Chain Concept and Its Chemiosmotic Consequences." *Science* 206: 1148–1159.

——1981. ''Biochemical Mechanism of Proton Motivated Phosphorylation in $F_0$ $F_1$ Adenosine Triphosphatase Molecules.'' C.P. Lee, Gottfried Schatz, and Gustav Dallner (eds.), *Mitochondria and Microsomes*. Reading, MA: Addison-Wesley, pp. 427–457.

——1985. ''Molecular Mechanics of Protonmotive $F_0F_1$ ATPases.'' *FEBS Letters* 182: 1–7.

——1991. ''Foundations of Vectorial Metabolism and Osmochemistry.'' *Bioscience Reports* 11: 297–346.

Monod, Jacques, Changeux, Jean-Pierre, and Jacob, François. 1963. ''Allosteric Proteins and Cellular Control Systems.'' *Journal of Molecular Biology* 6: 306–329.

Monod, Jacques, Wyman, Jeffries, and Changeux, Jean-Pierre. 1965. ''On the Nature of Allosteric Transitions: A Plausible Model.'' *Journal of Molecular Biology* 12: 88–118.

Negelein, Erwin, and Brömel, Heinz. 1939. ''R-Diphosphoglycerinsäure, Ihre Isolierung und Eigenschaften.'' *Biochemische Zeitschrift* 303: 132–144.

Orgel, Leslie. 2000. ''Are You Serious, Dr. Mitchell?'' *Nature* 402: 17.

Penefsky, Harvey S., Pullman, Maynard E., Datta, A., and Racker, Efraim. 1960. ''Partial Resolution of the Enzymes Catalyzing Oxidative Phosphorylation. II Participation of a Soluble Adenosine Triphosphatase in Oxidative Phosphorylation.'' *Journal of Biological Chemistry* 235: 3330–3336.

Perutz, Max. 1970. ''Stereochemistry of Cooperative Effects in Haemoglobin.'' *Nature* 228: 726–734.

——1998. *I Wish I'd Made You Angry Earlier. Essays on Science, Scientists and Humanity*. Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Planck, Max. 1949. *A Scientific Autobiography*, trans. F. Gaynor. New York: Philosophical Library.

Prebble, John. 2000. ''The Lasting Value of Mitchell's Mechanisms.'' *Nature* 404: 330.

——2001. ''The Philosophical Origins of Mitchell's Chemiosmotic Concepts.'' *Journal of the History of Biology* 34: 433–460.

——2002. ''Peter Mitchell and the Ox Phos Wars.'' *Trends in Biochemical Sciences* 27: 209–212.

——2010. ''The Discovery of Oxidative Phosphorylation: A Conceptual Off-Shoot from the Study of Glycolysis.'' *Studies in History and Philosophy of Biological and Biomedical Sciences* 41: 353–362.

Prebble, John, and Weber, Bruce. 2003. *Wandering in the Gardens of the Mind*. New York: Oxford University Press.

——2008. ''Peter Mitchell: Changing the Face of Bioenergetics.'' Oren Harman and Michael R. Dietrich (eds.), *Rebels, Mavericks and Heretics in Biology*. New Haven: Yale University Press, pp. 147–231.

Pullman, Maynard E., Penefsky, Harvey S., Datta, A., and Racker, Efraim. 1960. ''Partial Resolution of the Enzymes Catalyzing Oxidative Phosphorylation. I. Purification and Properties of Soluble Dinitrophenol-Stimulated Adenosine Triphosphatase.'' *Journal of Biological Chemistry* 235: 3322–3329.

Racker, Efraim. 1965. *Mechanisms in Bioenergetics*. New York: Academic Press.

——1970. ''Function and Structure of the Inner Membrane of Mitochondria and Chloroplasts.'' Efraim Racker (ed.), *Membranes of Mitochondria and Chloroplasts*. New York: Van Nostrand Reinhold, pp. 127–171.

Racker, Efraim, and Stoeckenius, Walther. 1974. ''Reconstitution of Purple Membrane Vesicles Catalyzing Light-Driven Proton Uptake and Adenosine Triphosphate Formation.'' *Journal of Biological Chemistry* 249: 662–663.

Reid, Robert, Moyle, Jennifer, and Mitchell, Peter. 1966. "Synthesis of Adenosine Triphosphate by a Proton Motive Force in Rat Liver Mitochondria." *Nature* 212: 257–258.

Rheinberger, Hans-Jörg. 1997. *Toward a History of Epistemic Things. Synthesizing Proteins in the Test Tube*. Stanford: Stanford University Press.

Slater, Edward C. 1953. "Mechanism of Phosphorylation in the Respiratory Chain." *Nature* 172: 975–978.

——1974. "From Cytochrome to Adenosine Triphosphate and Back." *Biochemical Society Transactions* 2: 1149–1163.

——1976. "Bioenergetics: Past, Present and Future." Arthur Kornberg, Bernard L. Horecker, L. Cornudella, and J. Oro (eds.), *Reflections on Biochemistry*. Oxford: Pergamon Press, pp. 45–55.

——1994. "Peter Dennis Mitchell." *Biographical Memoirs of Fellows of the Royal Society* 40: 281–305.

University of Wisconsin. 1942. *Symposium on Respiratory Enzymes*. Madison: University of Wisconsin Press.

Warburg, Otto, and Christian, Walter. 1939. "Isolierung und Kristallisation des Proteins des Oxydierenden Garungsferments." *Biochemische Zeitschrift* 303: 49–68.

Weber, Bruce, and Prebble, John. 2006. "An Issue of Originality and Priority: The Correspondence and Theories of Oxidative Phosphorylation of Peter Mitchell and Robert J.P. Williams, 1961–1980." *Journal of the History of Biology* 39: 125–163.

Williams, Robert J.P. 1961. "Possible Functions of Chains of Catalysts." *Journal of Theoretical Biology* 1: 1–17.

Yasuo, Kagawa, Sone, Nobuhito, Yoshida, Masasuke, Hirata, Hajime, and Okamoto, Harumasa. 1976. "Proton Translocating ATPase of a Thermophilic Bacterium. Morphology, Subunits and Chemical Composition." *Journal of Biochemistry* 80: 141–151.