

Reflections

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Keilin, Cytochrome, and the Respiratory Chain

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The defining episode in my scientific career was my close association with David Keilin in the nearly 10 years (1946–1955) during which I was a member of the Molteno Institute at the University of Cambridge. Not only did my work in Cambridge determine the direction of my subsequent research, but Keilin's character, way of working and thinking, and his integrity as a scientist and human being were a continuing inspiration. I remained in contact with him until his death, and one of my proudest moments was when, during his first venture abroad since the War for the conferring of an honorary degree in Utrecht, I was able to show him my laboratory in Amsterdam.

David Keilin (1887–1963)

David Keilin was born in Moscow on March 21, 1887, of Polish parentage; his father was a businessman and small landowner. The family returned to Warsaw where he graduated from the Górski High School in 1904. He studied medicine at the University of Liège in Belgium for a year, but in 1905, being advised that his health would not stand the strain of medical studies, moved to Paris to study biology. In 1915, he obtained his doctorate with a thesis on the biology of insect larvae. In the same year he was invited by G. H. F. Nuttall to be his assistant at the Quick Laboratory of Parasitology in Cambridge, England, the forerunner of the Molteno Institute, where he was to spend the rest of his working life. He was appointed Lecturer in Parasitology in 1925 and in 1931 succeeded Nuttall as Professor and Director of the Molteno Institute. He had to relinquish both posts in 1952 upon reaching the compulsory retirement age of 65 but was able to continue working in the Institute until his death in 1963. He received many honors, including election as Fellow of the Royal Society in 1928 and the award in 1952 of the highest honor of the Society, the Copley Medal. Many do not understand why he was never awarded the Nobel Prize.

Keilin's paper in the *Proceedings of the Royal Society* in 1925 with the title "On cytochrome, a respiratory pigment, common to animals, yeast, and higher plants" (1) marked the beginning of studies of what Warburg later called the respiratory chain (atmungskette), many of us called the electron transfer chain, and David Green, with some prescience, the electron transport chain. The story of how Keilin came upon cytochrome when studying hemoglobin in the horse intestinal parasite *Gastrophilus intestinalis* is told in his posthumously published book (2).

Already 75 years ago there was quite a lot known about biological oxidations. The word "oxidase" had already been introduced by Gabriel Bertrand (3) in the 19th century to describe the enzyme responsible for the hardening of lacquer, now known as laccase. In 1910–1912 Battelli and Stern made thorough studies of the oxidation of a number of substances by oxygen in the presence of ground-up tissue and showed the sensitivity of this process to cyanide (4). They referred to the enzyme responsible as indophenol oxidase from the color reaction they used to measure its activity. In the early 1920s, Thunberg (5) showed that the oxidation of a large number of organic compounds such as succinic acid is catalyzed by enzymes, each specific for its substrate, named dehydrases and later dehydrogenases by Wieland (6). As is well



FIG. 1. David Keilin (1887–1963).

known, a controversy developed concerning the mechanism of biological oxidations. Wieland and Thunberg, impressed by the ability of dehydrogenases to catalyze the oxidation of organic compounds by artificial acceptors such as methylene blue, proposed that the fundamental action is the activation by the dehydrogenases of hydrogen atoms, otherwise inert, so that they can react with oxygen. Warburg, impressed by the presence of iron in respiring cells and the ability of cyanide both to combine with iron and to inhibit cell respiration, proposed that the fundamental process is the activation of oxygen by an iron-containing respiratory enzyme (atmungsferment) (7).

Keilin's paper made it clear that the electrons derived from the activation of the hydrogen atoms by the dehydrogenase are transferred via three hemoproteins, which he named cytochromes *a*, *b*, and *c*, to an oxygen-activating oxidase. He did not name the oxidase in his 1925 paper, but in 1927 identified it, on the basis of its sensitivity to cyanide, with Battelli and Stern's indophenol oxidase and on the basis of its sensitivity to both cyanide and carbon monoxide with Warburg's atmungsferment (8). Much to Warburg's chagrin he continued to call it indophenol oxidase and, in retaliation perhaps, Warburg refused to accept the role of the cytochromes. This became one of the controversies of the 1930s (see Ref. 9), matching the vigorous confrontations in this field 30 or 40 years later at the annual meetings of the ASBC.

The basic features of our present picture of the respiratory chain were established by Keilin and his co-workers in the 1920s and 1930s. Already in his first paper, he showed that cytochrome *b* is the first acceptor of electrons from substrate. Making use of the exceptional stability of cytochrome *c*, Keilin and Hartree (10) extracted it from heart muscle. Most importantly, in 1939 (11) they showed that what had hitherto been thought of as a single cytochrome *a* consists of two components that they now called cytochromes *a* and *a*₃. In contrast to the other cytochromes, including cytochrome *a*, cytochrome *a*₃ combines with carbon monoxide and cyanide and has, therefore, all the properties ascribed to Warburg's atmungsferment.

By 1939, it was possible to write the respiratory chain as a simple chain: dehydrogenase → cytochrome *b* → cytochrome *c* → cytochrome *a* → cytochrome *a*₃ → oxygen. Strictly speaking the order of cytochrome *c* → cytochrome *a* had not been established (it could have been reversed), but Keilin was convinced, correctly as it is now known, that cytochromes *a* and *a*₃ are closely associated.

Expansion of Keilin's Respiratory Chain

That was still the situation when in 1946 I joined Keilin as a Ph.D. student (rather mature in age by English standards but not in biochemical knowledge (see Ref. 12)), and more than a half-century later, this description of the respiratory chain is still valid although additional electron-transferring components have been added to it. The first of these was cytochrome c_1 , already discovered by Okunuki in 1939 (13) but not generally accepted until Keilin and Hartree in 1955 showed that the absorption band initially ascribed to cytochrome c is derived from two components, one the classical cytochrome c and the other Okunuki's cytochrome c_1 (14).¹

The second addition to Keilin's respiratory chain, proposed in 1948 before cytochrome c_1 was accepted, was an electron-transferring factor acting in the chain between cytochromes b and c that was irreversibly and specifically destroyed by aerobic incubation with a dithiol compound, called BAL (17). After the discovery by Van Potter that the powerful respiratory chain inhibitor antimycin also inhibits electron transfer between cytochromes b and c , which he ascribed (incorrectly as it transpired) to its binding to the factor, he kindly gave it the name Slater factor (18).

In the late 1950s, F. L. Crane (19) in David Green's laboratory discovered ubiquinone (coenzyme Q) as a new hydrogen carrier between the dehydrogenases and the electron transfer chain proper, but it was not until much later that it was recognized that ubiquinone is also involved in electron transfer within the respiratory chain (see below).

After many earlier proposals that copper is involved as well as iron in the oxidation of cytochrome c , this was finally established in the 1960s by Helmut Beinert, using paramagnetic resonance spectrometry (EPR) (20). Bob van Gelder (21) in my Amsterdam laboratory showed that the cytochrome c oxidase takes up 4 electrons per molecule, one each into the hemes of cytochromes a and a_3 and two into the copper atoms.²

The application by Beinert of EPR spectrometry revealed also a whole new class of electron carriers, the iron-sulfur centers (22). With one exception, these centers are involved in the transfer of reducing equivalents from the flavin, by then recognized as a component of all ubiquinone-reducing dehydrogenases, to ubiquinone, rather than in Keilin's respiratory chain itself. The one exception was not in fact discovered by Beinert but by his colleague Rieske and is generally known as the Rieske iron-sulfur protein (23). The high redox potential, around about that of cytochrome c_1 , made it an attractive site of action of antimycin and a candidate for my old factor. However, there was no experimental evidence for a reaction with antimycin, and for many years in Amsterdam we did not know quite what to do about the Rieske protein until Simon de Vries found that its EPR spectrum is affected by ubiquinone (24). The breakthrough was made after Bernie Trumpower (25) showed that, after extraction of the Rieske protein, antimycin inhibits the *reduction* of cytochrome b , instead of its *oxidation*, as it was supposed to do if it inhibits the chain between cytochromes b and c . This reminded me of an old observation by Deul and Thorn (26) in my laboratory that this is exactly what antimycin does after destruction of the factor, what we called the "double kill" experiment. Sure enough Simon de Vries showed that the treatment I had used in the 1940s to destroy the factor has a drastic effect on the EPR spectrum of Rieske's iron-sulfur protein (27). By establishing the identity of my factor and the Rieske protein, the number of possible components of the respiratory chain was at least reduced by one.

The double kill experiment is nicely explained by Mitchell's Q cycle (26) to which I had paid insufficient attention when it was proposed, despite a friendly letter from Peter saying that it would give him great pleasure if it turned out that the Q cycle explained the Slater factor. It does. According to this cycle, there are two possible entries of electrons from ubiquinol to cytochrome b , one coupled with the reduction of the Rieske iron-sulfur protein and therefore susceptible to BAL treatment and one via a separate antimycin-sensitive ubiquinol-binding site, which (when the cycle is functioning) operates in the opposite direction by accepting electrons from cytochrome b . I soon became an enthusiastic supporter of the ubiquitous Q cycle (29).

¹ To my everlasting embarrassment, I had published a paper in 1949 (15) in which I concluded that Okunuki's evidence for the existence of cytochrome c_1 was unsatisfactory (see also Ref. 16).

² That it was much later shown that cytochrome c oxidase contains 3 atoms of copper per molecule is not inconsistent with van Gelder's titrations, because two of the copper atoms are coupled and take up only a single electron.

I have now got a bit ahead of myself chronologically. Just as is the case with Keilin's *c* and *a* absorption bands, the *b* band turned out also to be double, but in this case it is derived from two protoheme prosthetic groups bound to a single polypeptide chain. The first clue of the existence of two components came from Britton Chance and was established in his laboratory in a redox titration by Wilson and Dutton (30). There was quite a lot of what turned out to be rather cloudy work on cytochrome *b* in the 1970s, but the dust settled with Fred Sanger's determination of its molecular weight via DNA (31), which told me that it is a two-heme cytochrome (32). Its function was established by the Q cycle as a transmembrane subunit of ubiquinol-cytochrome *c* reductase with the lower potential heme, denoted b_{566} , accepting electrons from ubiquinol on the outside of the inner membrane and transferring them to the higher potential heme (b_{562}) on the inside of the membrane and eventually to ubiquinone.

Fractionation of the Respiratory Chain

Keilin and his students used for their studies of the respiratory chain a suspension of small particles obtained by grinding heart muscle with sand in weak phosphate buffer that became known as the Keilin and Hartree heart muscle preparation (33). I do not think that much attention was given in early studies to the nature or origin of these particles. Indeed I think that I was the first to show that they contain about 30% lipid, an accidental observation made when I was looking for a method of determining the dry weight of the preparation, since in those days the activity of a respiratory preparation was expressed by the Q_{O_2} (μl of O_2 /h/mg, dry weight). When as a newcomer I asked Ted Hartree how to measure the dry weight of the suspension in the buffer, he suggested that I precipitate it with trichloroacetic acid, centrifuge, wash the precipitate, dry it, and weigh it. This I did, but I decided to speed up the drying process by washing with ethanol. I found that this decreased the weight by 30%, compared with washing with water, and changed my definition of Q_{O_2} to base it on fat-free dry weight. I did observe that the ethanol extract was bright yellow but did not give this any thought, thereby missing the opportunity of discovering ubiquinone.

The significance of the lipid became clear when at about this time Albert Claude (34) showed that the site of intracellular respiration is the mitochondrion and, when the mitochondrion was viewed by thin section electron microscopy by Palade (35), more precisely in the inner membrane or cristae. We now recognize that the Keilin and Hartree preparation consists of submitochondrial particles, or vesicles, derived from the inner membrane.

No attempt was made by the Keilin school to fractionate the chain apart from the isolation of cytochrome *c*. The first success was obtained by Wainio (36) and Lucile Smith (37) using deoxycholate and cholate, respectively, to disperse the membrane and allow its components to be separated by conventional ammonium sulfate fractionation. David Green's school (38) importantly expanded this technique to the separation of what he called four complexes, catalyzing, respectively, the reduction of ubiquinone by NADH (Complex I) or succinate (Complex II), the reduction of ferricytochrome *c* by ubiquinol (Complex III), and the oxidation of ferrocycytochrome *c* by oxygen (Complex IV). I have always thought it a pity that he gave the name Complex to these multisubunit proteins, each of which has a clearly defined enzyme function.

Function of the Respiratory Chain

In the 1920s Keilin and Warburg envisaged that the function of the respiratory chain is to catalyze the oxidation of intermediary metabolites by the transfer of electrons derived from hydrogen atoms to oxygen. That it might have an additional function in ion transport was suggested in 1939 by Lundergårdh (39), specifically that in plants the cytochromes act as electron carriers in one direction and as anion carriers in the opposite direction. The primary function of the respiratory chain, oxidative phosphorylation, was discovered by Engelhardt in 1931 (40). Measurements of the stoichiometry (P:O ratio), made independently in 1939–1940 by Belitzer and Tsibakowa (41) in Leningrad in the USSR and Severo Ochoa (42) in Oxford in England, established that phosphorylation must be coupled not, or not only, to the dehydrogenation of substrate but to electron transfer along the respiratory chain.

Oxidative Phosphorylation and Topography of the Respiratory Chain

After completing my Ph.D. in 1948 with a thesis on the succinate oxidase system and a subsequent study of the NADH oxidase system (43), oxidative phosphorylation was the logical next topic for my research, especially after Al Lehninger's paper on oxidative phosphorylation

coupled to the oxidation of NADH (44). This was a new field for the Molteno Institute, and given the opportunity by the Rockefeller Foundation to study in the United States, I spent about 6 months working in Severo Ochoa's laboratory at New York University learning the new techniques.³ Severo's interests were then mainly on carbon dioxide fixation, but in the same building, Ef Racker was developing the concept of an acyl intermediate in the oxidative phosphorylation reaction of glycolysis (46). Adapting an enzyme assay that he had described, I developed a procedure that enabled me to measure oxidative phosphorylation between substrate and cytochrome *c* (47), the first direct demonstration of what later became known as "site 2 oxidative phosphorylation." I continued these studies after returning to the Molteno Institute and in 1953 published what became known as the "chemical hypothesis" of oxidative phosphorylation (48) in which, by analogy with substrate-linked phosphorylation, the energy of electron transfer is conserved primarily in non-phosphorylated high energy forms of components of the chain.

Around this time, the two functions of the respiratory chain, ion movements and oxidative phosphorylation, were beginning to coalesce. Workers on gastric secretion favored a simple redox pump mechanism, according to which the secreted protons were those liberated from hydrogen carriers by transfer of electrons to the cytochromes. In 1951, however, both salt accumulation in plants (49) and gastric secretion (50) were found to be inhibited by 2,4-dinitrophenol, known to uncouple oxidative phosphorylation from electron transfer. This focused attention on ATP instead of electron transfer as the source of charge separation, and Davies and Krebs (51) proposed in 1951 that "ionic concentration differences, *i.e.* osmotic energy . . . may play a role in the synthesis of ATP." Williams (52) proposed that protons could bring about condensation reactions such as polyphosphate formation.

These concepts were developed by Mitchell (53) into a coherent hypothesis encompassing a functional link between electron transfer in the respiratory chain and the translocation in the opposite direction of protons across the inner mitochondrial membrane, whereby the energy is conserved as an electrochemical proton gradient. To accommodate experimental evidence of an $H^+ : e$ ratio of about 2, in 1966 he introduced the concept of loops in the respiratory chain with two electrons crossing the membrane from one side to the other followed by two hydrogen atoms in the opposite direction (54). This very important concept of the sidedness of the membrane with the specific location of the electron acceptors and donors was not at first generally accepted, not only because many did not at first accept (or possibly understand) the precise role of protons envisaged by Mitchell but also because those of us more specifically interested in the respiratory chain knew that the order of electron transfer originally proposed was wrong. Mitchell's brilliant proposal of the Q cycle (28), made in answer to these criticisms, as a description of how the oxidation of ubiquinol by ferricytochrome *c* is coupled to the net production of protons on one side of the membrane and their consumption on the other side was soon given solid experimental support.

As more became known of the structure of the two large multisubunit proteins involved in the respiratory chain, namely ubiquinol-cytochrome *c* reductase and cytochrome *c* oxidase, as well as of the ubiquinone-reducing dehydrogenases, such as succinate-ubiquinone reductase and NADH-ubiquinone reductase, it became clear that their dimensions are in fact greater than a phospholipid bilayer and that they are embedded and specifically orientated across the phospholipid layer, which confirmed in structural terms Mitchell's sidedness concept.

Mechanism of Electron Transfer

The discovery in the 1960s and early 1970s of more and more electron-transferring centers in the respiratory chain, particularly the multiplicity of iron-sulfur centers, gave a lot of headaches to those of us who found even the 1948 sequence of dehydrogenase $\rightarrow b \rightarrow$ factor $\rightarrow c \rightarrow a \rightarrow a_3 \rightarrow O_2$ longer than necessary to accommodate a P:O ratio of 3 in oxidative phosphorylation. At the time of the International Congress in Switzerland in 1970, I remember that, in desperation, we proposed double chains.

The real function of the multiplicity of electron transfer centers has only recently become understood as a result of the structural information that tells us where the centers are located in the protein, together with a fundamental increase in our understanding of the nature of electron transfer. Dutton and co-workers (55) have demonstrated that, by virtue of electron

³ A biographical note on Severo Ochoa is to be found in Arthur Kornberg's "Reflections" (45).

tunneling, electrons can readily travel through the protein medium a distance of up to 14 Å between redox centers but that transfer over greater distances is facilitated by a chain of electron carriers. Within this distance of 14 Å, rapid electron tunneling takes place even if the electron transfer is endergonic, provided that the centers are sufficiently close. It is the proximity of the redox centers in chains that provides highly directional electron transfer.

The role of distance between the redox centers in controlling the rate and therefore the specificity of electron transfer is beautifully illustrated by the mobility of the Rieske iron-sulfur protein subunit in ubiquinol-cytochrome *c* reductase, as shown by the x-ray crystallographic studies of Berry, Crofts, and their colleagues (56). In one conformation, stabilized by the ubiquinol inhibitor stigmatellin, the Fe-S cluster is close enough to the ubiquinol-binding site to allow its reduction by ubiquinol. In a second conformation, it is close enough (about 8 Å) to the heme in cytochrome *c*₁ to permit rapid electron transfer. The important point is that in neither conformation can both reactions occur at a suitable rate. For example, in the stigmatellin-stabilized conformation, the iron-sulfur cluster is about 27 Å from the heme. Thus, the reaction mechanism must involve movement of the Rieske iron-sulfur protein. Keilin would have enjoyed this paper. X-ray crystallography of proteins was not strange to him. He supported Kendrew and Perutz in their work and lived to see the solution of the structures of myoglobin and hemoglobin.

We now know that one of Keilin's cytochromes, cytochrome *b*, as well as Okunuki's cytochrome *c*₁, are subunits of a single protein, ubiquinol-cytochrome *c* reductase, and that his cytochromes *a* and *a*₃ are also bound to a single subunit of cytochrome *c* oxidase. Cytochrome *c* remains a single polypeptide. In his earlier papers, Keilin often used the singular "cytochrome" to refer to the cytochrome system, and I think that he regarded them as acting as a single unit. In 1947, he and Hartree stated that "the catalysts in the particles, as in the intact cells, are more or less rigidly held together in a framework that assures their mutual accessibility and a consequent high catalytic activity" (33). This idea of an ordered macromolecular assembly, under the name of the "solid state" model of the respiratory chain, seems to be winning favor over the "liquid state" model that envisaged independent free diffusion of the multisubunit proteins in the membrane and of cytochrome *c* in the space between the inner and outer membranes of the mitochondrion (see *e.g.* Ref. 57).

In any case, the function of the cytochromes is to transfer electrons. It is the function of the protons, freed by this removal of the electrons from the hydrogen atoms of intermediary metabolites, to drive ion transport and the synthesis of ATP. As Mitchell pointed out in the conclusion to his Nobel Lecture in 1978, "David Keilin's chemically simple view of the respiratory chain appears now to have been right all along." (58).

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REFERENCES

1. Keilin, D. (1925) *Proc. R. Soc. Lond. B Biol. Sci.* **98**, 312–339
2. Keilin, D. (1966) *The History of Cell Respiration and Cytochrome*, Cambridge University Press, Cambridge, UK
3. Bertrand, G. (1897) *Ann. Chem. (Phys.)* **12**, 115–140
4. Battelli, F., and Stern, L. (1910) *Biochem. Z.* **30**, 172–194
5. Thunberg, T. (1917) *Skand. Arch. Physiol.* **35**, 163
6. Wieland, O. (1912) *Ber. Dtsch. Chem. Ges.* **45**, 484–499; 2606–2615
7. Warburg, O. (1924) *Biochem. Z.* **177**, 471–486
8. Keilin, D. (1927) *Nature* **119**, 670–671
9. Slater, E. C. (1977) *Trends Biochem. Sci.* **21**, 156–157
10. Keilin, D., and Hartree, E. F. (1937) *Proc. R. Soc. Lond. B Biol. Sci.* **122**, 298–308
11. Keilin, D., and Hartree, E. F. (1939) *Proc. R. Soc. Lond. B Biol. Sci.* **127**, 167–191
12. Slater, E. C. (2003) *IUBMB Life* **55**, in press
13. Yakushiji, E., and Okunuki, K. (1940) *Proc. Imp. Acad. Tokyo* **16**, 299–302
14. Keilin, D., and Hartree, E. F. (1955) *Nature* **176**, 200–206
15. Slater, E. C. (1949) *Nature* **163**, 532
16. Slater, E. C. (1986) in *Selected Topics in the History of Biochemistry (Comprehensive Biochemistry)* (Semenza, G., ed) Vol. 36, pp. 197–253, Elsevier Science Publishers B.V., Amsterdam
17. Slater, E. C. (1948) *Nature* **161**, 405–406
18. Potter, V. R., and Reif, A. E. (1952) *J. Biol. Chem.* **204**, 287–297
19. Crane, F. L., Hatefi, Y., Lester, R. L., and Widmer, C. (1967) *Biochim. Biophys. Acta* **25**, 220–221
20. Beinert, H., and Palmer, G. (1964) *J. Biol. Chem.* **239**, 1221–1227
21. van Gelder, B. F. (1966) *Biochim. Biophys. Acta* **118**, 36–46
22. Beinert, H., and Sands, R. H. (1960) *Biochem. Biophys. Res. Commun.* **3**, 41–46
23. Rieske, J. S., Hansen, R. E., and Zaugg, W. S. (1964) *J. Biol. Chem.* **239**, 3017–3022
24. de Vries, S., Albracht, S. P. J., and Leeuwerik, F. J. (1979) *Biochim. Biophys. Acta* **546**, 316–333
25. Trumpower, B. L. (1976) *Biochem. Biophys. Res. Commun.* **70**, 73–78
26. Deul, D. H., and Thorn, M. B. (1962) *Biochim. Biophys. Acta* **59**, 426–436

27. Slater, E. C., and de Vries, S. (1980) *Nature* **288**, 717–718
28. Mitchell, P. D. (1975) *FEBS Lett.* **59**, 1–6
29. Slater, E. C. (1983) *Trends Biochem. Res.* **8**, 239–242
30. Wilson, D. F., and Dutton, P. L. (1970) *Biochem. Biophys. Res. Commun.* **39**, 59–64
31. Anderson, S., Bankier, A. T., Barrell, B. C., De Bruijn, M. H. L., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. F., Roe, D. P., Sanger, F., Schreier, P. R., Smith, A. J. H., Staden, R., and Young, I. G. (1981) *Nature* **290**, 457–465
32. Slater, E. C. (1981) in *Chemiosmotic Proton Circuits in Biological Membranes* (Skulachev, V. D., and Hinkle, P., eds) pp. 69–104, Addison-Wesley, Reading, MA
33. Keilin, D., and Hartree, E. F. (1947) *Biochem. J.* **41**, 500–502
34. Hogeboom, G. H., Claude, A., and Hotchkiss, R. D. (1946) *J. Biol. Chem.* **165**, 615–629
35. Palade, G. E. (1952) *Anat. Rec.* **114**, 427
36. Wainio, W. W., Cooperstein, S. J., Kollen, S., and Echel, B. (1948) *J. Biol. Chem.* **173**, 145
37. Smith, L., and Stotz, E. H. (1948) *J. Biol. Chem.* **208**, 819
38. Green, D. E. (1966) in *Comprehensive Biochemistry* (Florkin, M., and Stotz, E. H., eds) Vol. 14, pp. 309–326, Elsevier Science Publishers B.V., Amsterdam
39. Lundergårdh, H. (1939) *Nature* **143**, 203
40. Engelhardt, W. A. (1930) *Biochem. Z.* **227**, 16–38
41. Belitzer, V. A., and Tsibakowa, E. T. (1939) *Biokhimiya* **4**, 516
42. Ochoa, S. (1940) *Nature* **146**, 267
43. Slater, E. C. (1950) *Biochem. J.* **46**, 484–499
44. Friedkin, M., and Lehninger, A. L. (1948) *J. Biol. Chem.* **174**, 757–758
45. Kornberg, A. (2001) *J. Biol. Chem.* **276**, 3–11
46. Racker, E. F. (1961) *J. Biol. Chem.* **190**, 685–696
47. Slater, E. C. (1950) *Nature* **166**, 982–984
48. Slater, E. C. (1953) *Nature* **172**, 975–978
49. Robertson, R. N., Wilkins, M. J., and Weeks, D. C. (1951) *Aust. J. Sci. Res. B* **4**, 248–264
50. Davies, R. E. (1951) *Biol. Rev.* **26**, 87–120
51. Davies, R. E., and Krebs, H. A. (1951) *Biochem. Soc. Symp.* **8**, 77–92
52. Williams, R. J. P. (1949) in *The Enzymes* (Boyer, P., Lardy, H., and Myrbäck, H., eds) Vol. 1, pp. 391–441, Academic Press, New York
53. Mitchell, P. (1961) *Nature* **191**, 144–148
54. Mitchell, P. (1966) *Biol. Rev.* **41**, 455–602
55. Page, C. C., Moser, C. C., Chen, X., and Dutton, P. L. (1999) *Nature* **402**, 47–52
56. Zhang, Z., Huang, L., Schulmeister, V. M., Chi, Y. I., Kim, K. K., Hung, L. W., Crofts, A. R., Berry, E. A., and Kim, S. H. (1998) *Nature* **392**, 677–684
57. Schägger, H. (2002) *Biochim. Biophys. Acta* **1555**, 154–159
58. Mitchell, P. (1979) *Les Prix Nobel en 1978*. The Nobel Foundation, 1979, pp. 134–172, Almqvist Wiksell International, Stockholm