Chapter 4
Struggling with the Standard Model (1930–1941)

In the early 1930s, photosynthesis research was still far from being the popular theme that it would later become. Those working in this field did so in an almost intimate atmosphere, having few potential collaborators—or competitors—as colleagues; and most of them were closely interrelated by friendship, personal collaborations or teacher–student relationships. The main work in photosynthesis research was undertaken in only a small number of places: in Berlin (Germany), in Pasadena and Chicago (USA) and in Cambridge (UK).[1] And it was only a small number of scientists, who studied the subject over a prolonged period of time, among these the main protagonists of this chapter: William Arnold, Robert Emerson, Charles Stacy French, Hans Gaffron and Robert (Robin) Hill. Others became engrossed in the field, although originally they had intended to take a quick research-opportunistic look at the subject before returning to their original interests; these included, most prominently, James Franck, and to some extent Hans Kautsky and Cornelis B. van Niel. The social structure of this group of actors—distributed over different places but in constant communication with each other—makes it very amenable to an analysis of heuristic strategies of a community.

If one were to single out a common feature of the photosynthesis experiments carried out in this period, it would be, in reception of Otto Warburg's work, the application of the technique of manometry to the study of the unicellular green alga *Chlorella*. Many of the scientists listed above, who would become world experts in photosynthesis, spent an extended period of research at Warburg's laboratory in the Dahlem district of Berlin, where they became familiar with the technique and with the model organism. Emerson went to Warburg's laboratory to write his doctoral thesis, and he continued to make use of the technical knowledge that he acquired in Dahlem for the rest of his life. He also transmitted this know-how to all his students, together with the conviction that there was no better alternative. French spent a year of his postdoctoral research in Berlin, having being sent there by Emerson, his mentor at the time. Gaffron worked for several years with Warburg, interrupted only

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[1] In addition to these, the universities at Berkeley and at Urbana–Champaign in the USA would soon—in the 1940s—become equally important centres of photosynthesis research.

K. Nickelsen, *Explaining Photosynthesis,* History, Philosophy and Theory
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by a research stay at Caltech, where he also visited Emerson. Franck never worked directly with Warburg, but they nevertheless knew each other well from the time Franck spent in Berlin, where he had been one of Emil Warburg's students. Thus, out of all the main characters of this chapter only Hill, Kautsky and van Niel had no direct links with Warburg.

As the setting's actors were so closely intertwined, it was difficult to organise the material of this chapter in thematic sections; inevitably a number of arbitrary breaks were introduced. I start with a discussion of the establishment of a new standard model of the photosynthetic mechanism, which became the common reference point of this chapter's players. Its development is closely connected to Franck's entry into the field of photosynthesis research, which was prompted, among other things, by the use of chlorophyll fluorescence as a new way of investigating the photosynthesis mechanism. The contributions Franck made to extending the Willstätter–Stoll–Warburg model of photosynthesis (see Chapter 3) helped make the latter the "received view" of photosynthesis in this decade. The remainder of the chapter then contains the various challenges to the standard model that arose during this decade, and discusses how the different actors reacted to them. Inevitably, again, the description of the different models is rich in chemical detail, while reflective and summarising sections are inserted to help understand the general course of events, even if the technical passages are skipped.

4.1 Fluorescence and the Standard Model

4.1.1 The Kautsky Effect

Beginning in 1931, the German chemist Hans Kautsky, at the University of Heidelberg, began to approach the problem of how photosynthesis works from a new angle: he investigated the fluorescence of chlorophyll solutions, that is, the emission of light after the pigments had absorbed radiation.[2] This first quantitative and systematic study of the fluorescence of chlorophyll was part of a larger project to investigate the energy transformation processes on boundary layers (*Grenzflächen*) of the cell, and had far-reaching consequences on further developments in the field. The results were unexpectedly complex: when photosynthesising cells were illuminated, the fluorescence intensity (starting from a rather low level) rose sharply to a high transient state, and then, after a few seconds, it slowly decreased again until it reached a steady-state level. (This phenomenon would later become known as the "Kautsky effect").[3] Kautsky and his group were the first chemists to interpret systematically the resulting data in terms of an underlying mechanism. This

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[2] On Kautsky's life and work, see, e.g., von Gerhard (2004).

[3] See Kautsky and Hirsch (1931) and Kautsky et al. (1932) for the first reports of these phenomena, while Govindjee (1995) provides a historical review of the "Kautsky effect".

approach was based on the observation that, although chlorophyll solutions usually exhibited an intensive and beautifully red fluorescence, this fluorescence was found to decrease (to be "quenched", as it is called today) when the chlorophyll acted as a sensitiser in photochemical reactions, that is, transferred absorbed light energy to other molecules. Accordingly, Kautsky and his co-workers found that the fluorescence of assimilating leaves was comparably low, and that inhibiting photosynthesis with hydrogen cyanide resulted in a strong rise in fluorescence. Thus, fluorescence served as a convenient indicator of the efficiency of the cell's photosynthetic activity: the higher the fluorescence, the lower the utilisation of photons in photosynthesis.

In order to explain the curious rise of fluorescence at the onset of illumination, Kautsky and his co-workers suggested the following sequence of reaction steps: when illumination started, the fluorescence intensity was low because all the absorbed energy could be transferred to an acceptor molecule in the system. However, the concentration of this molecule almost immediately dropped again, which resulted in the peak in fluorescence (since the energy absorbed by the chlorophyll could not be transferred). The rapid increase in fluorescence was neither influenced by temperature nor by the addition of cyanide, and was thus taken to reflect a purely photochemical process. According to Kautsky, the subsequent slow decrease in fluorescence indicated that in this phase the chlorophyll transferred its energy again to an acceptor molecule in the system, the concentration of which rose very slowly. Since in this phase the rate of reaction was strongly influenced by both temperature and cyanide, as well as being linked to a strong rise in oxygen production, Kautsky and his co-workers suggested that, in parallel to the transfer of light energy, a thermochemical catalytic reaction was taking place, which produced oxygen.[4] At the same time, the group thought that oxygen was the molecule to which the chlorophyll transferred the absorbed light energy. A detailed theory of "sensitised photooxidation" was developed, which involved an activated, metastable state of oxygen that was particularly apt to oxidise further molecules in its surroundings. This process was purported to be at the core of the photochemical events that occurred during photosynthesis.[5]

Once it had been discovered that fluorescence studies were related to the photosynthesis problem, another eminent physicist, James Franck, joined the scene and tried to explain Kautsky's observations within the framework of the Willstätter–Stoll–Warburg model of photosynthesis.

Govindjee (2004a) covers the phenomenon of chlorophyll *a* fluorescence from both a historical and a systematic viewpoint.

[4] See Kautsky and Hirsch (1931) and Kautsky et al. (1932).

[5] See Kautsky et al. (1932, 1933, 1935). This proposal was contested by Hans Gaffron, who argued that photosynthesis started without oxygen; see Gaffron (1935). It was presumably through his follow-up of this debate that James Franck first became acquainted with Gaffron's work.

4.1.2 James Franck and Photosynthesis

James Franck is usually remembered for his seminal contributions to physics *sensu stricto* rather than for his work in photosynthesis.[6] Franck started his academic career as a doctoral student of Emil Warburg in Berlin, where he received his doctoral degree in 1906. Despite the difficulties that academics of Jewish origin were experiencing at that time, Franck stayed in Berlin and pursued his scientific interests as an assistant to the experimental physicist Heinrich Rubens, who had succeeded Emil Warburg at Berlin's Friedrich Wilhelm University. In 1911 Franck was promoted, on acceptance of his habilitation thesis, to the status of *Privatdozent* (which is roughly equivalent to the rank of an associate professor but without a proper salary). From 1912 to 1914, Franck collaborated with Gustav Hertz, another of Rubens's assistants and nephew of the renowned physicist Heinrich Hertz. The celebrated paper that arose from their collaborative work confirmed Planck's 1900 quantum hypothesis by showing that electrons scattering on a gas of mercury atoms lost energy only in quantised amounts. This "discovery of the laws governing the impact of an electron upon an atom" earned Franck and Hertz the 1925 Nobel Prize in Physics.[7] However, the beginning of the First World War, which placed other themes on their agendas, brought this fruitful collaboration to an end. In 1921, Franck accepted the Chair of Experimental Physics at the University of Göttingen (Germany), where he spent twelve highly productive years. Franck's focus of interest slowly shifted to the problems of energy exchange in photochemistry, in particular to the phenomena of fluorescence, phosphorescence and chemiluminescence.[8] With hindsight, this work paved the way for Franck's later interest in the physical foundations of photosynthesis. (Incidentally, it was also at this time that Eugene Rabinowitch became Franck's private research assistant: Rabinowitch was another of those physicists who would catch the "photochemical bug" and would eventually be drawn into the world of photosynthesis research).

Franck's happy years in Göttingen abruptly ended after the Nazi Government came to power in 1933. Following the infamous "Law for the Restoration of the Professional Civil Service", issued on 7 April 1933, all persons with at least one Jewish grandparent were dismissed from the civil service, which included university academics. And although Franck, as a First World War veteran, would have fallen under the only exemption clause to this law, he publicly resigned from his

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[6] On Franck's life and work see, e.g., the biographical memoir by Kuhn (1965) and the tribute by Rosenberg (2004). Beyerchen (1996) analyses Franck's emigration from Germany and its consequences, notably his scientific migration to photosynthesis research. See also the extensive biography by Lemmerich (2007).

[7] Quote taken from the Nobel Prize Announcement at http://www.nobelprize.org/nobel_prizes/physics/laureates/1925/. See Franck and Hertz (1914) for the pertinent publication. Hon and Goldstein (2013) provides a lucid account of the discovery.

[8] This work included Franck's well-known paper on the "elementary processes of photochemical reactions", an analysis of the shape of molecular absorption and fluorescence spectra, which includes what later became known as the Franck–Condon principle; see Franck (1925).

professorship at Göttingen in protest. This courageous step caused an enormous stir, nationally and internationally, among scientists, politicians and the wider public.[9] The consequences were far-reaching. Although Franck had originally intended to stay in Germany, he soon realised that he would be unable to find a new academic post or a position in industry in his home country as long as the political circumstances did not change. Thus, after a short stay at the Johns Hopkins University in Baltimore, Franck spent a year at Niels Bohr's institute in Copenhagen (Denmark). In the meantime a professorship at Johns Hopkins had been arranged for him, which he was able to accept in 1935.

It was during these first years of exile that Franck became interested in the photochemical aspects of photosynthesis. In a detailed study of Franck's emigration from Germany, and his coincidental migration to a different field of science, the historian Alan Beyerchen identified Franck's stay in Copenhagen as the crucial turning point.[10] Franck's role there, as envisaged by Bohr, was to pursue current problems in nuclear physics. However, Franck became increasingly unhappy with this function: he found the field of nuclear physics too crowded, while his access to appropriate resources was too limited for him to be able to compete on an equal footing. Instead, Franck began a project on chlorophyll fluorescence of green leaves with Hilda Levi, a young molecular spectroscopist.[11] In addition, Franck collaborated again with Rabinowitch, who had in the meantime also emigrated to Copenhagen.[12] In an interview with Levi, Beyerchen learned that Franck became involved only because chlorophyll made good fluorescing solutions, which could be used to study the underlying energy exchange processes. At the time these processes, in particular the mechanism of sensitised photooxidation, were the subject of highly controversial debates.[13] However, Franck must have developed a genuine interest in photosynthesis shortly thereafter, since in the very same issue of the journal *Naturwissenschaften*, in which he published his findings with Levi, Franck also published his first conceptual paper on the photochemical mechanism of photosynthesis.[14]

A little later, Franck left Copenhagen and took up the tenured position in Baltimore. However, since he found working in nuclear physics equally unsatisfactory there, Franck continued the line of physico-chemical research that he had begun while in Copenhagen—and he would keep to the photochemistry of green plants for the rest of his working life. In his "Remarks on Photosynthesis" (1935), Franck

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[9] An English translation of the pertinent documents (as well as perceptive commentaries and useful background information) can be found in Hentschel (1996, pp. 21–34).

[10] Beyerchen (1996, pp. 77–79).

[11] See Franck and Levi (1935a, b) for the resulting publications.

[12] See Franck and Rabinowitch (1934), in which they formulated the hypothesis of the "cage effect", based on their investigation of the photolysis of different compounds.

[13] Beyerchen (1996, p. 80). Beyerchen refers to an interview that he conducted with Hilda Levi on 12 November 1980 in Copenhagen.

[14] See Franck (1935a).

presented his ideas to the English-speaking world for the first time;[15] while in the following decades Franck published a series of increasingly sophisticated physico-chemical photosynthesis models, which he developed with various co-authors.[16] In view of this new field of research of his, Franck was invited, in 1938, to set up a laboratory dedicated to the study of photosynthesis at the University of Chicago—a project that was financially supported by the Jewish philanthropist Samuel Fels. Franck would direct the Fels Laboratory until his retirement in 1949. Thereafter, he was succeeded by his longstanding co-worker and friend Hans Gaffron, but even though he had given up the directorship, Franck continued to take an active part in the work carried out at the laboratory. Franck had invited Gaffron to come and work with him in Chicago in 1939 and, as Franck's former collaborator and biographer Jerome Rosenberg wrote, "the two constituted an interesting complementary pair, one emphasizing physical mechanisms, and the other comparative biochemistry and plant physiology".[17]

Although Franck started his photosynthesis studies as a typical research opportunist (he intended to have a shot at this theme, based on the expertise he had gathered in other fields, and then move on to other subjects again), events took a different turn. In a talk delivered at the Franck Memorial Symposium in 1966, Gaffron claimed that Franck had admitted that, by opting for photosynthesis, he had got more than he had bargained for: "His fate resembled that of the man who curiously puts a finger on a strip of flypaper, does not succeed in shaking it off and winds up in a terrible mess. In Franck's case this mess was biochemistry".[18] In the same vein, (the aforementioned) Rabinowitch, one of Franck's most ardent admirers, described Franck's entry into the sphere of photosynthesis research:

> He thought that the confusion prevailing in this field was due to [the] lack of precise definition and controlled experimentation by biologists, and that the quantitative approach of a physicist would soon dispel it. But he did not reckon with the complexity of phenomena in living cells. Franck believed that each measurement must mean something in biology, as it does in physics, and can be used as a reliable stone in constructing a mechanism or formulating a theory. The trouble is that in biology, no experiment can be "controlled" in the full sense this term has in physics, because the state and the properties of a living cell depend on its whole history, and thus on more variables than can be reliably controlled.[19]

Franck himself came to acknowledge the unforeseen difficulties: "It differs fundamentally from physics", he wrote to his close friend and former colleague Lise Meitner in 1941; "there, the most simple solution nearly always is correct, but this is

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[15] See Franck (1935b).

[16] See Franck and Herzfeld (1937); Franck et al. (1941); Weller and Franck (1941); Franck (1945, 1949). Franck's final attempt to solve the problem was completed shortly before his death: see Franck and Rosenberg (1964).

[17] Rosenberg (2004, p. 73).

[18] Quoted in Beyerchen (1996, p. 82).

[19] Quoted in Beyerchen (1996, pp. 82–83).

absolutely not the case in living material".[20] On the other hand, only a few of Franck's colleagues in the field of photosynthesis research were able to grasp the gist of his contributions—first and foremost because they lacked the necessary background in physics, but also because, at the time, few people were interested in the details of the primary photochemical process to which Franck had turned his attention. In the end, most of his work on photosynthesis was superseded. However, Franck brought more to photosynthesis than his personal theories: He raised questions from the point of view of a physicist that drew attention to lines of research that were not sufficiently appreciated by his fellow biochemists and physiologists. Franck's outspoken goal was to make his colleagues realise that all models of the mechanism of photosynthesis had to meet the fundamental laws of physics—even though this would mean to discard some of their biological pet hypotheses.[21]

4.1.3 The New Standard Model

4.1.3.1 Stoll and Willstätter Again

Not only Franck developed a strong interest in the findings presented by Kautsky and his group but also Arthur Stoll and later Richard Willstätter made another attempt to solve the problem of how chlorophyll acted in photosynthesis, based on Kautsky's observations. Confirming their earlier suggestions,[22] Stoll, in 1932, reported his finding that the hydrogen atoms at position 9 of the chlorophyll molecule were very loosely bound, so that the chlorophyll could easily and reversibly be dehydrogenated. This made it probable, Stoll maintained, that chlorophyll played the role of both hydrogen donor and acceptor in photosynthesis.[23] While Stoll repeated his and Willstätter's earlier assumption that chlorophyll was able to transfer hydrogen to an activated derivative of carbonic acid, bound to the central magnesium atom of chlorophyll, he now considered more precisely the actual origin of this hydrogen: namely water. Chlorophyll, Stoll surmised, might be able to decompose water under the influence of light, possibly according to the equation: $2\ H_2O \longrightarrow 2\ H + H_2O_2$. Stoll suggested that the hydrogen released in this process would hydrogenate the chlorophyll, thereby raising the latter to a higher state of hydrogenation than usual. And in order to prevent the hydrogen peroxide, which was formed during the decomposition of water, from immediately dehydrogenating the chlorophyll again, the peroxide had to be decomposed to water and oxygen. This, Stoll stated, would match the earlier finding by him and Willstätter that a temperature-dependent process

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[20] Franck to Meitner, quoted in Lemmerich (2007, p. 238); original German.

[21] Cf. Franck (1935b, p. 433).

[22] Cf. Willstätter and Stoll (1918).

[23] Stoll (1932, p. 957).

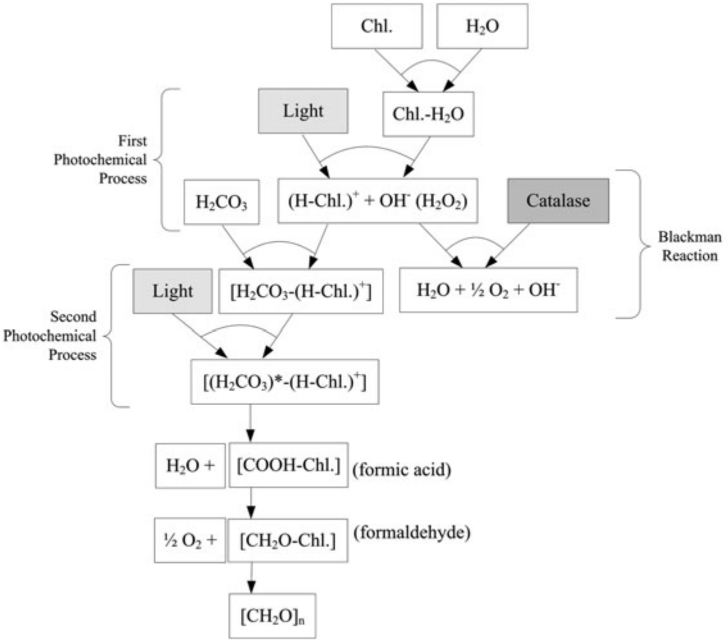

Fig. 4.1 The extended model of photosynthesis proposed by Stoll (1932).

occurred during photosynthesis, which most probably involved an enzyme similar to catalase, and which prompted the decomposition of hydrogen peroxide.[24]

Figure 4.1 shows this new photosynthesis model in a graph form. Water binds to chlorophyll, forming the complex Chl-H_2O. The latter is decomposed under the influence of light, whereby chlorophyll is hydrogenated to H-Chl. This is the first photochemical process. The simultaneously produced OH radicals would, most probably, combine to form hydrogen peroxide, which would immediately be removed under the influence of the enzyme catalase, whereupon oxygen is released. This was interpreted to be the temperature-dependent, enzymatic Blackman reaction. Hydrogenated chlorophyll (H-Chl) then binds carbonic acid (H_2CO_3) to form a complex. Under the influence of light, the carbonic acid in this complex would be activated (which in the graph is indicated by a star) and transformed into a derivative that is susceptible to reduction: this is the second photochemical reaction. A hydrogen then is transferred from chlorophyll to the activated carbonic acid derivative, which yields an unstable intermediate (H_3CO_3-Chl) that immediately decomposes to a derivative

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[24] This was in line with the general belief at the time that the Blackman reaction (according to its kinetics) consisted of a reaction between catalase and a peroxide. Warburg and Uyesugi (1924) was particularly influential in this respect. It was only in Emerson and Green (1937) that the supposed similarity between the Blackman reaction and the reaction between catalase and hydrogen peroxide was contested.

bound to chlorophyll (H_2CO_2-Chl) and a hydroxyl radical (OH). The further steps of the process were not entirely clear. Most probably the sequence of first and second photochemical reaction would be repeated: the chlorophyll of the complex would be hydrogenated again and the formyl derivative activated. Thereupon another hydrogen transfer (and loss of oxygen) would eventually yield a formaldehyde derivative chlorophyll complex (H_2CO-Chl), from which the formaldehyde was released that would undergo condensation reactions to form glucose. Stoll argued that this mechanism was in close agreement with the findings of Kautsky and his group: in the dark periods, Stoll assumed, chlorophyll was bound to both water and carbonic acid. At the onset of illumination the chlorophyll was able, in principle, to transfer the activated hydrogen to its acceptor (the carbonic acid derivative), while the latter still had to be formed. This delay in acceptor formation should cause the fluorescence to increase rapidly, until the hydrogen acceptor was available in sufficient quantities. However, Stoll disagreed with Kautsky's verdict that oxygen was the first hydrogen (or electron) acceptor—without, though, fully explaining his objections.

Unlike Stoll, Willstätter, his former mentor, accepted the involvement of oxygen and integrated it into a model, which was published in 1933.[25] In this model, reconstructed in a graph form in Fig. 4.2, oxygen was, in fact, needed for photosynthesis to take place, namely for the dehydrogenation of chlorophyll: oxygen oxidised chlorophyll (that is, it took away one of the chlorophyll's loosely bound hydrogen atoms), which resulted in the formation of monodehydrochlorophyll (Chl^{DH}) and the radical O_2H. However, as this form of chlorophyll was considered unstable, it would be rapidly rearranged to the completely (di-)dehydrogenated form of chlorophyll (Chl^{DDH}) by donating the second loosely bound hydrogen to its central $\mathrm{Mg}(H_2CO_3)$ complex (Willstätter also took it for granted that carbonic acid would bind to the chlorophyll's magnesium):

$$\mathrm{Chl.} + O_2 \longrightarrow O_2H + \mathrm{Chl.}^{DH}$$
$$\mathrm{Chl.}^{DH} + CO_3H_2 \longrightarrow [CO_3H_2 + H] + \mathrm{Chl.}^{DDH}$$

The $\mathrm{Chl.}^{DDH}$ thus formed was thought to react, under the influence of light, with water, whereby hydroxyl radicals and $\mathrm{Chl.}^{DH}$ were formed. Again, the latter donated the loosely bound hydrogen to the centrally bound carbonic acid:

$$\mathrm{Chl.}^{DDH} + H_2O \xrightarrow[\text{light}]{} OH + \mathrm{Chl.}^{DH}$$
$$\mathrm{Chl.}^{DH} + [CO_3H_2 + H] \longrightarrow [CO_3H_2 + 2H] + \mathrm{Chl.}^{DDH}$$

Willstätter identified the latter as the central photochemical reaction, which was repeated three times, so that altogether four hydrogen atoms were transferred to the central magnesium complex. This sufficed for the complete reduction of the carbonic acid molecule. Thereafter (that is, as soon as the magnesium complex was fully saturated and carbonic acid completely reduced), $\mathrm{Chl.}^{DH}$ would, in a reaction with water molecules, be reduced to ordinary chlorophyll again. Willstätter did not go into any detail about the fate of the reduced carbon moiety, but one can safely

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[25] Willstätter (1933).

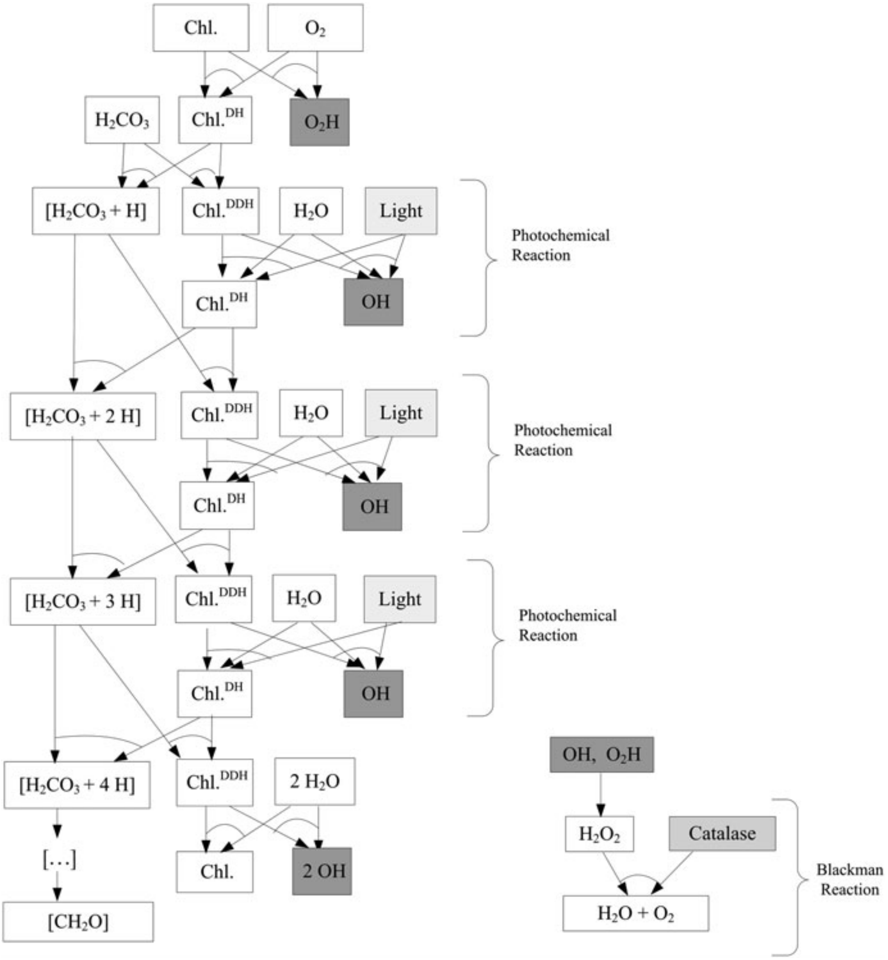

Fig. 4.2 Willstätter's photosynthesis model (1933).

assume that he believed that it was reduced to carbohydrates via formaldehyde in the usual way. All the radicals that were produced in the process (OH, O_2H) were assumed to end up as hydrogen peroxide, which was decomposed in the Blackman reaction through the action of the enzyme catalase.

Thus, in contrast to Stoll, who thought that, in photosynthesis, chlorophyll acted in a *higher state of hydrogenation*, namely as H-Chl., Willstätter assumed that it was the *dehydrogenated forms* of chlorophyll that entered the photochemical reaction. Both scientists, however, introduced the possibility that water might be decomposed in the course of photosynthesis and might donate hydrogen to the chlorophyll, which was then transferred to the carbonic acid bound to the central magnesium atom of chlorophyll. And both of them were convinced that the thermochemical Blackman reaction consisted of the decomposition of hydrogen peroxide through catalase, as a result of which molecular oxygen was released. It is interesting to see, though, that this "module" (since as such it was treated by both) was integrated very differently into the two divergent options. Neither of these suggestions contested the earlier Willstätter–Stoll model; rather, one of the partial processes (in this case, the light-driven reduction of carbonic acid or its derivative by the action of chlorophyll) was singled out and modelled in more detail than before—triggered, among other things, by the new empirical results of Kautsky's group and by Stoll's finding that the structure of chlorophyll has two weakly bound hydrogen atoms. Thus, both suggestions are classic examples of a model being locally "extended".

4.1.3.2 Franck Joins the Field

This was the state of affairs at the time that Franck published his first contribution to photosynthesis studies in 1935.[26] He conceded that, in 1918 and then in their contributions of 1932 and 1933, Willstätter and Stoll had offered:

> … strong evidence that chlorophyll not only acts as a sensitizer, but that it enters into the course of the chemical reactions. Chlorophyll, having two especially loosely bound hydrogen atoms, is assumed to give off these atoms in reducing carbon dioxide and to regain the hydrogen by dissociating water.[27]

However, given his background in theoretical photochemistry, Franck was not satisfied with the prevailing suggestions for the underlying mechanism. Franck's main argument was that the steps proposed by Willstätter as being the core of the photochemical process were energetically impossible if one took for granted that for each step one quantum of red light was available (as was generally assumed to be the case, based on the findings by Warburg and Negelein of 1923; see section 3.3). Franck was equally dissatisfied with Kautsky's explanation of the course of chlorophyll fluorescence, which, Franck argued, implied assumptions that were at odds with the body of general knowledge of the fluorescence of liquids.

Hence, Franck presented an alternative mechanism, which not only met the energetic requirements but also explained why monodehydrochlorophyll (Chl.^{DH}) was necessary for the process to start (on this matter Franck agreed with Willstätter); and why the intensity of fluorescence was such a complicated function of irradiation time. Franck's model suggestion is reconstructed in a graph form in Fig. 4.3. In his paper, Franck emphasised that the following conditions had to be met:

> (i) If 4 quanta are necessary to reduce one carbon dioxide molecule, four different photochemical reactions have to be considered, since storing up energy in the form of the excitation energy of molecules is impossible. Hypotheses about metastable states with a long life time were likewise ruled out because the reactions took place in a condensed system.

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[26] Franck (1935a, b).

[27] Franck (1935b, p. 433).

(ii) For each photochemical partial reaction the energy of 1 quantum of red light had to suffice.
(iii) Each individual photochemical step had to take place with a yield of unity, in accordance with the total quantum yield. Therefore, only those photochemical partial reactions could be considered in which at least one of the products was not a radical, so that back reactions would not take place.[28]

Franck believed that the last condition in particular dealt a final blow to Willstätter's 1933 proposal, which required the involvement of several radicals (such as OH, O_2H). This was far too costly, energetically speaking, given that Warburg and Negelein had determined the minimum quantum requirement of the process as four to five. Stoll's assumption that hydrogenated forms of chlorophyll might be involved was likewise refuted by Franck, since he believed that these compounds were too unstable to play a major role. Four photochemical steps had to be found, each of which required no more energy than was provided by one quantum of red light: this was, from Franck's perspective, the principal challenge. As can be taken from his papers, Franck found it almost impossible to devise a photosynthesis pathway that was sufficiently parsimonious in terms of energy expenditure. In his attempt to solve this task, Franck assumed that first monodehydrochlorophyll (Chl.^{DH}) was formed under the influence of light:

$$\text{Chl.} \underset{\text{light}}{\longrightarrow} \text{Chl.}^{DH} + \text{H}$$

(This reaction, Franck maintained, was the reason for the induction period of photosynthesis, which had repeatedly been observed; at the same time it explained the rapidly appearing peak in fluorescence that Kautsky had reported). If no oxygen was present, the initial state of the chlorophyll would be quickly restored by the reverse reaction; while in the presence of oxygen the hydrogen atom would be used up in the following processes:

$$\text{H} + \text{O}_2 \longrightarrow \text{O}_2\text{H}$$
$$\text{O}_2\text{H} + \text{O}_2\text{H} \longrightarrow \text{H}_2\text{O}_2 + \text{O}_2$$

The hydrogen peroxide (H_2O_2) would then be removed by the action of catalase, in agreement with the earlier suggestion made by other authors. However, according to Franck the main procedure consisted of a series of reactions that occurred in and around the chlorophyll molecule, in which hydrogen atoms were exchanged for OH radicals. Concurring with Willstätter and Stoll, Franck assumed that these exchange reactions took place in a complex of chlorophyll and carbonic acid (or one of its derivatives), which went through the stages of formic acid and formaldehyde. The formaldehyde then was the usual starting point for the formation of carbohydrates in condensation reactions. If illumination was stopped, the monodehydrochlorophyll (Chl.^{DH}) would be restored to the usual form of chlorophyll (Chl.) by taking up a hydrogen atom from formic acid or formaldehyde (which would destroy some of the light reaction products). Franck was also ready to assume that, instead of oxygen, other primary hydrogen acceptors might possibly be involved in the first

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[28] Cf. Franck (1935b, p. 436).

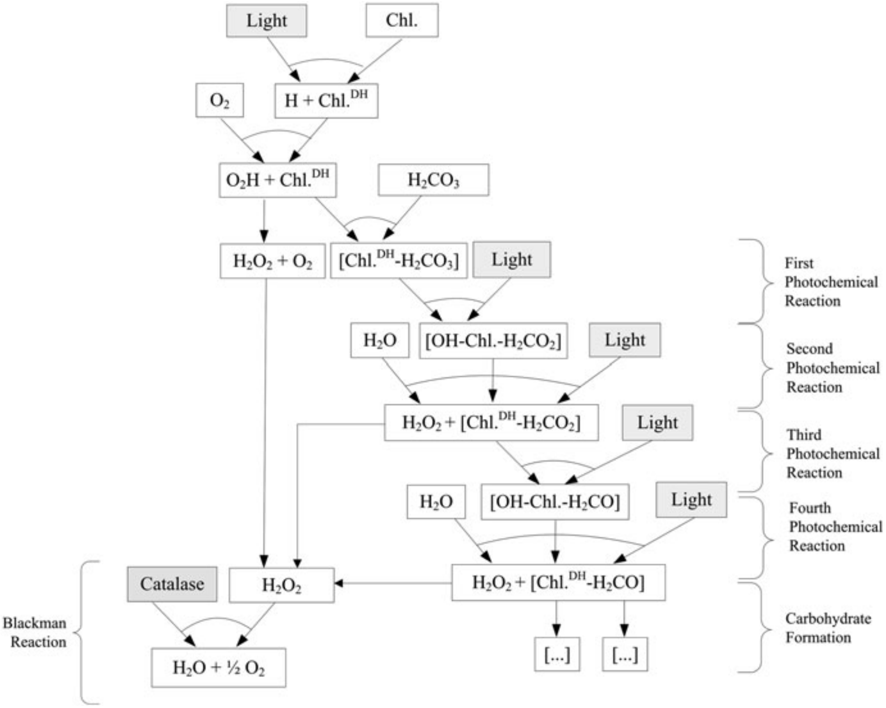

Fig. 4.3 Franck's model of photosynthesis in 1935.

step of the process (since Hans Gaffron had shown that photosynthesis also occurred in anaerobic conditions, without any oxygen present), although these supplements would be less effective: "The result of a lack of oxygen would then be that the induction period is lengthened", Franck concluded.[29]

Franck's suggestion, which is reconstructed in a graph form in Fig. 4.3, became widely accepted as the new "standard model" of photosynthesis. It was a modified and extended version of the original Willstätter–Stoll model, in which the photochemical and biochemical steps were adapted to the Warburg–Negelein value of the energy requirements of the process (4 light quanta per one molecule of oxygen). The fact that this adaptation was possible was seen to strongly support the general Willstätter–Stoll approach. Chlorophyll was still considered to be the site *and* the agent of photochemically driven carbon dioxide reduction, and oxygen was thought to be released from carbon dioxide, via the decomposition of hydrogen peroxide by catalase. The latter seemed to be the enzymatic, thermochemical Blackman reaction, while light acted in a series of reactions that took place in a complex of chlorophyll

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[29] Franck (1935b, p. 437).

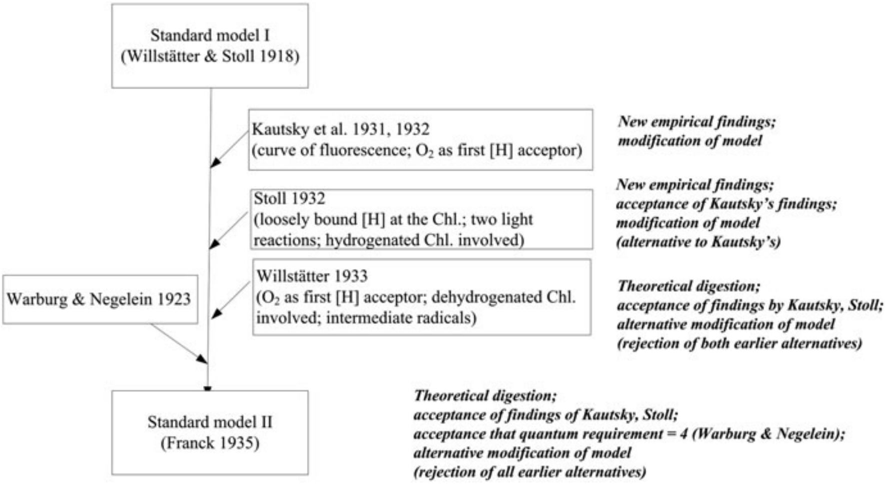

Fig. 4.4 From the first standard model (Willstätter and Stoll 1918) to the second standard model (Franck 1935).

and carbonic acid (or derivatives). Everything seemed to be in place and settled—were it not for the experimental findings published by Robert Emerson and William Arnold in 1932. Franck had not taken their findings seriously (neither had many of his colleagues); yet with hindsight it seems that Franck's model was already outdated by the time it was published. However, before I turn to these experiments, I shall briefly consider the different contributions so far from a systematic point of view.

4.1.3.3 Reflective Summary

A rough sketch of the relationship between the different proposals is given in Fig. 4.4. The first input considered in this chapter was of an *empirical* nature: based on a new methodical approach, Kautsky and his group presented their new finding, namely the curious shape of the fluorescence curve, which indicated the existence of underlying processes that had so far not been explained by the standard model. Kautsky's group thus suggested that molecular oxygen had to be integrated into the standard model of photosynthesis as the first hydrogen acceptor. Kautsky did not, however, present an extended model suggestion himself but left this task to others.

Kautsky's work was examined by Stoll, who was not convinced that oxygen was the first hydrogen acceptor, although he did admit that, in order to explain Kautsky's fluorescence curve, the standard model needed to be modified. From his own studies, Stoll reported another empirical finding, namely that chlorophyll has two loosely bound hydrogens, which (from Stoll's point of view) supported the assumption that it acted not only as a sensitiser but also took part in the actual redox reactions in photosynthesis. Thus, in his modification of the model, Stoll assumed that metastable, hydrogenated states of chlorophyll were involved and that two photochemical reactions took place (one of which would provide the energy for reducing the carbon dioxide in the chlorophyll complex; while the other, newly suggested reaction was thought to decompose water molecules, which Stoll regarded as a possible hydrogen donor). Stoll retained the earlier assumption that the Blackman reaction consisted of the decomposition of peroxides through the effect of catalase.

This suggestion was rejected, only one year later, by Stoll's former mentor and colleague Willstätter, who had no new empirical findings to add, but had thoroughly digested the earlier findings from a theoretical point of view. Willstätter accepted that the new empirical findings (of Kautsky, of Stoll and also of Warburg and Negelein) had to be accommodated by a modified photosynthesis model *and* he also regarded Kautsky's suggestion—that oxygen might be the first acceptor—as plausible. Furthermore, Willstätter assumed that the photochemical process consisted of several cycles of partial reactions, all of which involved radicals. Subsequent reactions also required the involvement of radicals; likewise, the Blackman reaction was associated with the reaction between catalase and hydrogen peroxide.

Both Stoll and Willstätter still considered their earlier approach to be "by and large" accurate; they retained the central elements such as the chlorophyll–carbon dioxide complex, the path of sugar formation via formaldehyde and the enzymatic dark reaction that yielded oxygen. The interpretation of the latter as the catalase reaction, removing hydrogen peroxides under oxygen release, had been added in the 1920s to the standard body of knowledge. Rather, their goal was to refine certain aspects of the model, while leaving other parts untouched. For example, they disagreed on where hydrogen peroxide was produced in the process, in what kind of state the chlorophyll would react and, most importantly, what exactly the photochemical reaction of photosynthesis consisted of.

Franck's contribution of 1935 marked the final digested state of the model. Franck also accepted the new empirical findings, but he took the 1923 finding of Warburg and Negelein—that photosynthesis required only 4 quanta of red light to produce one molecule of oxygen—far more seriously than the others had done. The quantum requirement, in fact, was taken by Franck to be the central parameter to which all adequate photosynthesis models had to adhere. This restriction made it highly improbable that metastable states of the chlorophyll molecule or radicals of any kind were involved in the reaction (as both Stoll and Willstätter had assumed), while Franck accepted Kautsky's suggestion that molecular oxygen was a primary hydrogen acceptor. Franck also retained the concept of the catalase reaction, and he did not even question the synthesis of sugars via formaldehyde. The resulting model hypothesis was as conservative as possible, as explanatory as possible (in view of the available empirical evidence) and as innovative as necessary (in suggesting a central sequence of four photochemical reactions in a series that did not include radicals). It was a well-balanced attempt to save the phenomena that had been established thus far and, at the same time, the generally accepted photosynthesis model.

4.2 The Crucial Experiments of 1932

With hindsight, the models of Kautsky, Stoll, Willstätter and Franck were only passing phenomena—while they were highly debated at the time and nicely illustrate how researchers struggled, around 1930, to reconcile new empirical findings, established bodies of knowledge and acknowledged theoretical requirements. By contrast, one of the most important developments of lasting impact of the early and mid-1930s was the concept of a "photosynthetic unit", which originated from the 1932 experiments carried out by Robert Emerson and William Arnold.

They found, in the course of flashing light experiments on photosynthesis, that, even under optimal conditions, only one molecule of oxygen was evolved in the alga *Chlorella* per about 2400 molecules of chlorophyll.[30] This result was quite unsettling, because up to then it had been taken for granted that every chlorophyll molecule would be as active as the other in binding carbon dioxide and, subsequently, reducing it. Emerson and Arnold had no idea how to make sense of this finding. It was only in 1936 that Hans Gaffron and Kurt Wohl would provide a theoretical interpretation and coin the actual term "photosynthetic unit"—but even then, this interpretation was not immediately well received. In the following, I shall first provide some background information on Emerson, who was the senior researcher of this project and is also a major figure in later chapters, after which I shall proceed to the 1932 experiments and examine how they were interpreted.

4.2.1 Robert Emerson: Harvard, Berlin, Caltech and Stanford

Robert Emerson dedicated his entire professional career to the study of photosynthesis with manometric methods, and his findings profoundly influenced and promoted this field of research.[31] When Emerson first went to Harvard University in 1921, he studied animal physiology, with the intention of (eventually) becoming a doctor. However, his interest soon shifted from animals to plants. Emerson himself ascribed this change of mind primarily to the influence of the botanist and plant physiologist Winthrop J. V. Osterhout, who took on Emerson as his laboratory assistant.[32] Osterhout is regarded as one of the founders of general physiology, which was the label

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[30] The experiments and the many difficulties in realising the set-up have been described many times; see Myers (1994); Arnold (1991) and Govindjee (2001). Govindjee et al. (1996) is a special issue of the journal *Photosynthesis Research* dedicated to William Arnold; Govindjee (2014) provides a biography.

[31] The biographical information on Emerson has been taken from the memoir by Rabinowitch (1961), complemented by the details given in Govindjee (2004b), and by Emerson's own CV of 1936, which is held in his estate: *Curriculum vitae and bibliography of Robert Emerson*, Robert Emerson Papers, 1923–1961, Record Series 15/4/28, Box 1, University of Illinois Archives.

[32] Cf. Govindjee (2004b, p. 184). On Osterhout, see Blinks (1974).

given to an increasingly successful movement initiated by the eminent physiologist Jacques Loeb.[33] When Emerson was at Harvard, this new physiology was strongly promoted as an attractive alternative to the morphologically dominated curricula of traditional botany and zoology. Osterhout's research interests at the time were centred on the study of membrane properties, a subject to which he contributed some pioneering work; and he also worked on photosynthesis for a brief period.[34] Osterhout was one of the first professors at Harvard to integrate his own research and the recent work done by other scientists at other institutions into his lectures, which was rather unusual at the time. Furthermore, Osterhout gave a laboratory course that was for some time the only place at Harvard where students could undertake practical work in biochemistry.[35] With his engaged way of teaching, Osterhout succeeded in attracting many gifted students to his new experimental approach to studying life processes, Emerson being one of them.

Having received his first degree at Harvard in 1925, Emerson continued his graduate work in the country that was then the centre of science: Germany. He intended to study the formation of chlorophyll in plants, so he first planned to go to Munich and work with Willstätter, the leading chlorophyll expert of the time. However, since Willstätter had resigned his university position in 1924, as a public sign of protest against strong anti-semitic tendencies among faculty members, Willstätter advised Emerson to go and work with Otto Warburg in Berlin instead.[36] Emerson followed Willstätter's advice, and in 1927 he was awarded his doctorate from Berlin's Friedrich Wilhelm University.[37] It was during the course of his PhD studies and in this laboratory that Emerson became familiar with manometry and *Chlorella* as an experimental organism, both of which would play a significant role in the rest of his professional career. It was also in Berlin that Emerson first isolated the "Emerson strain" of *Chlorella pyrenoidosa*, which quickly became the standard experimental organism in photosynthesis research.[38]

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[33] On the development of general physiology and biochemistry in the USA around 1900, see Hall (1975); Kohler (1982) and Pauly (1987b). On Jacques Loeb, see Osterhout (1928); Pauly (1987a) and Fangerau (2010).

[34] Osterhout was the first to notice the induction period of photosynthesis and to attempt to study systematically the antagonism that exists between respiration and photosynthesis. See Osterhout (1918, 1919); Osterhout and Haas (1918, 1919).

[35] Blinks (1974, p. 224).

[36] Govindjee (2004b, p. 184). See also Willstätter's autobiography for background information on his resignation. Wiesen (2000) discusses the ambiguous reception of Willstätter's memoirs after 1945.

[37] The title of the thesis was (translated into English) "On the effect of hydrocyanic acid, hydrogen sulphide and carbon monoxide on the respiration of different algae". The thesis was officially handed in by the university's botanist Hans Kniep, which at first glance implies that Kniep was Emerson's supervisor. However, this (nominal) arrangement was due to the fact that only universities were authorised to award doctoral titles, while Kaiser Wilhelm Institutes and their members were not.

[38] See French (1959, p. 437).

Emerson then returned to Harvard, while in 1930 he moved to California, to take up the post of Assistant Professor of Biophysics at the California Institute of Technology (Caltech) in Pasadena. This position was part of a newly founded programme in biochemistry and biophysics, which, in structure and approach, closely resembled the departments in general physiology being established elsewhere. Besides Emerson, the group consisted of Henry Borsook, a biochemist who had also been trained in general physiology in Toronto (Canada),[39] and the plant physiologist Kenneth V. Thimann. This group of young and talented scientists was set up to work at the forefront of experimental biology.[40] However, in 1931 Emerson complained in a letter about the attitudes of the Caltech biologists, who were all "milk-bottle-molasses and beef-hash-muscle in outlook", while he found the biochemistry section too medical and "very narrow".[41] Emerson remained at Caltech until 1946, taking a leave of absence in the years 1937–1940, which he spent at the Carnegie Institution of Washington at the campus of Stanford University. From 1946 until his untimely death (in an aircraft crash in the East River, New York, on 3 February) in 1959, Emerson was Research Professor of Botany at the University of Illinois at Urbana–Champaign as well as the Director of the Photosynthesis Project there. Emerson succeeded in recruiting Rabinowitch as a second director, which resulted in one of the most productive and fruitful centres of photosynthesis research.

Emerson became one of the leading experts in photosynthesis. His painstaking accuracy in designing experiments and constructing set-ups are legendary; his research questions were to the point and his interpretations careful and convincing. Emerson's strategy was to specialise to the point of perfection: he hardly ever used a technique other than manometry; and he rarely worked on a theme that did not involve oxygenic photosynthesis in aquatic algae. This also was the experimental context of the crucial 1932 experiments, to which I shall turn in the next section.

4.2.2 Emerson, Arnold and 2500 Molecules of Chlorophyll

4.2.2.1 Setting the Stage

The crucial results of 1932 had their roots in Emerson's course in Plant Physiology at Caltech. William Arnold, an undergraduate student of physics, had ended up in Emerson's class, because he could not fit the obligatory course in Elementary Biology into his timetable. Emerson and Arnold, the professor and the student,

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[39] Borsook's recollections of this period are preserved in the interview carried out with him in 1978 by Mary Terrall, as part of the Caltech Archives Oral History Project; see Borsook (1978). On Thimann see, e.g., Stowe (1999).

[40] Kohler (1982, p. 318).

[41] Quoted in Kohler (1982, p. 322). Emerson to William J. Crozier, 24 March 1931. The original is held by the Harvard University Archives, Pusey Library: Crozier Papers. On Crozier and how he became a central figure in general physiology, see Pauly (1987b, in particular pp. 201–204).

who were only one year apart in age, took a liking to each other and engaged in scientific conversations that went far beyond the actual course work. As Arnold later recalled, Emerson was at the time very interested in the study of photosynthesis at intermittent light periods, studies that had been carried out by Brown and Escombe in 1905 and by Warburg in 1919 (see Chapter 3). The curious phenomenon was that one could omit as much as three-quarters of the light without there being a drop in the photosynthesis rate; and if conditions were optimised, one could actually increase the photosynthesis rate by using flashing light instead of continuous illumination. Emerson believed that these findings were important and considered adding a light source with rotating sectors to his own Warburg apparatus. Having heard this, Arnold suggested that Emerson might use neon lights for this purpose. Arnold was familiar with neon lights as a friend of his working in the Physics Department was involved in the development of these new light sources. Emerson agreed to Arnold's suggestion and he assured Arnold that installing the system would fulfil the laboratory work component of the Plant Physiology course. The experiment worked well, and when Arnold graduated in 1931, Emerson asked him to stay on and carry out some flashing light experiments with him. "Since I had been unable to find a place to do graduate work in astronomy, I agreed to continue as his assistant a while longer", Arnold explained later.[42] In the end, this stay would extend to another 15 months, and Arnold would never again return to either physics or astronomy.

Coming back to the 1932 experiments, the greatest difficulty the team had was building an appropriate flashing source and then implementing it into the manometric set-up. Arnold finally found that he could mount the neon tube on the water bath of the Warburg apparatus, directly underneath the vessels. This arrangement was eventually able to produce very short flashes of light. As usual, the rate of photosynthesis was measured manometrically. To ensure that the illumination was controlled only from underneath the reaction vessels, the sides and top of the vessels were silvered and then, to protect the silver, covered with copper jackets. The control vessels, which contained cultures grown in continuous light, were also illuminated by a bound neon tube mounted a few millimetres below the vessels. The only drawback was that the high precision necessary for equal illumination in all incidents only allowed them the use of a maximum set of three vessels at a time, two of which contained cell suspensions and one that was used as a zero control.

4.2.2.2 Separating the Photosynthesis Reactions

The first remarkable finding obtained using this set-up was that if the light period were sufficiently short and the dark period sufficiently long, photosynthesis rates could be increased by up to 400 %.[43] Emerson and Arnold's interpretation of this finding was in line with Warburg's earlier suggestion—that the photochemical reaction proceeded

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[42] Arnold (1991, p. 74).

[43] Emerson and Arnold (1932b, p. 417).

rapidly until an equilibrium concentration of its product was reached, which had to be removed by the thermochemical Blackman reaction before the next cycle could start. Emerson and Arnold believed, like many others at the time, that the Blackman reaction would release the chlorophyll from its complex binding to the carbonic acid derivatives, so that it would be able to react once more with carbon dioxide molecules.[44] The important achievement of this first paper of theirs was, however, that it provided the first realistic estimation of the time scale of a full cycle of photosynthesis. Emerson and Arnold maintained that "the dark reaction requires less than 0.04 seconds for completion at 25°C, and about 0.4 seconds at 1.1°C", while the light reaction, which was not affected by temperature, could take place in about a hundred-thousandth of a second.[45] These were numerical parameters almost as fundamental as the minimum quantum requirement, which all subsequent photosynthesis models had to accommodate.

4.2.2.3 The Photochemical Reaction in Photosynthesis

Emerson and Arnold published another paper in 1932, the scope of which was to establish the ratio between the number of chlorophyll molecules present in a cell suspension and the number of molecules of carbon dioxide that are reduced:

> From the experiments of Warburg and Negelein (1923), we know that the green alga *Chlorella pyrenoidosa* can reduce one molecule of carbon dioxide for each four quanta of light absorbed, when conditions permit maximum efficiency. Chlorophyll is clearly the substance absorbing the light quanta, so we may inquire how much chlorophyll must be present for the reduction of one molecule of carbon dioxide.[46]

If the photochemical reaction were saturated with light and the dark periods were long enough for the Blackman reaction to process all the photochemical products, then the number of carbon dioxide molecules reduced per light flash would reveal how many "units" of photosynthesis were present in the sample (the "unit" was regarded as an abstract entity, that is, as "the mechanism which must undergo the photochemical reaction to reduce one molecule of carbon dioxide".[47]) The chlorophyll content of the sample divided by the number of "units" would yield the number of chlorophyll molecules per unit. The background of this experiment was provided by a long-standing assumption, originally put forward by Willstätter and Stoll, that the rate of photosynthesis was independent of the chlorophyll content of a leaf, which Emerson had already challenged in an earlier paper, although he had not been able to clarify completely the relationship between chlorophyll and the rate of photosynthesis.

Their main technical problem was how they could produce flashes of sufficient light intensity to ensure light saturation. Emerson and Arnold finally succeeded by

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[44] See Emerson and Arnold (1932b, p. 418).

[45] Emerson and Arnold (1932b, p. 417).

[46] Emerson and Arnold (1932a, p. 191).

[47] Emerson and Arnold (1932a, p. 191).

concentrating the light incident on the cells by means of a concave mirror mounted below the neon tubes. Even then, light saturation was only approximated. They determined the chlorophyll content using a spectrophotometer, calibrated with standard samples of chlorophyll (the material for which, incidentally, had been provided by Hans Gaffron, who at the time was spending a brief period of research at Caltech). Algae cultures with varying chlorophyll content were grown by exposing them to light of different colours. However, more factors than previously suspected seemed to influence the rate of photosynthesis in the algae, as Emerson and Arnold admitted: "The chlorophyll concentration produced appears to depend on the intensity of the light and the age of the culture, as well as on the colour of the light. The neon light cultures mature faster than the incandescent light cultures, the mercury cultures much more slowly".[48] These observations proved to be typical: the complex behaviour of the cells, the performance of which was highly dependent on a plethora of environmental factors, would remain a challenge for all photosynthesis researchers using these organisms. Finding the optimal conditions for cellular growth and implementing these conditions as a standard became a central activity in all laboratories researching photosynthesis.

When the set-up was finally established, Emerson and Arnold found, to their utter surprise, a constant value of one molecule of oxygen evolved in *Chlorella* cells per about 2500 molecules of chlorophyll, which, consequently, had to be considered a "unit". Emerson and Arnold were as stunned as their audience and completely at a loss as to how to interpret this finding.[49] In the meantime, photosynthesis research was being decisively influenced by the developments taking place in a rather different field—one that, up to around 1930, nobody would have considered even remotely relevant to questions concerning photosynthesis: the discipline of microbiology. The driving force behind these developments was the Dutch microbiologist Cornelis B. van Niel; and his contributions will be examined before I turn to the conceptualisation of the photosynthetic unit.

4.3 The Generalised Equation for Photosynthesis

4.3.1 Cornelis B. Van Niel and General Microbiology

Cornelis B. van Niel (1897–1985)—or "Kees", as he was known to his friends—truly revolutionised the field of microbiology. In addition van Niel greatly advanced the field of photosynthesis research by bringing to the fore the fact that photosynthesis

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[48] Emerson and Arnold (1932a, pp. 193–194).

[49] The findings were confirmed two years later, by Arnold and Kohn (1934), who discovered, as stated in the abstract, that "in six species of plants, representing four phyla, the minimum number of chlorophyll molecules present for each molecule of carbon dioxide reduced appears to lie between 2000 and 3000".

occurs not only in plants but also in certain bacteria, and that the study of these organisms could contribute enormously to scientists' understanding of the workings of higher plants and algae.[50]

Van Niel's first degree of 1922 was in Chemical Engineering, which he studied at the then Delft Technical College in the Netherlands (which today is known as the Delft University of Technology). However, he found that microbial fermentation made up a large share of the curriculum of this subject, so that he switched to the Microbiology Department for his graduate studies. The department was then headed by Albert J. Kluyver, who had succeeded the eminent Martinus W. Beijerinck in this position—much to the surprise of his colleagues, since up to then Kluyver had been better known for his chemical expertise than for his knowledge of microbiology.[51] However, his appointment turned out to be most fortuitous: Kluyver became the founder of comparative microbiology, which soon made the college in Delft internationally famous. This tradition enormously influenced the young van Niel. Kluyver was convinced that the study of microbiology was highly relevant to a better understanding of the biology of higher organisms. His first microbiological paper, entitled "Unity and diversity in the metabolism of micro-organisms" (1924), was a comparative study within the bacterial realm. Only two years later, in 1926, Kluyver published, together with his associate Hendrick J. L. Donker, the classic and much more ambitious paper "Unity in Biochemistry".[52] In this paper (which, unfortunately, appeared in a rather obscure German journal), the authors proposed no less than a general theory of metabolism, aimed at unifying the study of biochemistry.[53]

In their 1926 paper Kluyver and Donker endorsed Heinrich Wieland's theory of redox reactions as being hydrogen transfers. (This notion was fiercely opposed by Warburg, who defended the view that oxygen had to be involved in oxidation reactions.[54]) Kluyver and Donker believed that hydrogen transfers were at the core of all metabolic reactions. From their point of view, even the most complicated biochemical processes could be reconstructed as a series of hydrogen transfer reactions; and Kluyver and Donker were able to provide ample evidence for this assumption from the realm of bacterial metabolism. This pointed perspective attracted much attention among fellow scientists; and Kluyver used his sudden popularity to promote comparative microbiology, which, he believed, deserved to become as widespread and influential as comparative anatomy once had been.[55]

In 1923, van Niel was made Kluyver's assistant and became responsible, from 1923 to 1928, for the large Delft culture collection of bacteria, yeasts, algae and protozoa. Van Niel thus had to familiarise himself thoroughly with the handling of a tremendous range of identified microbes, which he would later describe as

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[50] On van Niel see, e.g., Spath (1999); Barker and Hungate (1990) and Hungate (1986).

[51] See, on Kluyver's life and work, e.g., Woods (1957) and Kamp et al. (1959).

[52] Kluyver and Donker (1926). The original German title reads "Die Einheit in der Biochemie".

[53] On this paper's background and further implications, see Friedmann (2004).

[54] See Werner (1997) for an analysis of the controversy between Warburg and Wieland.

[55] See Spath (1999, Chapter 1, pp. 36–37).

having been a privilege. While carrying out this work, van Niel came across, for the first time, a group of purple bacteria that belonged to the family *Thiorhodaceae*, which was then the subject of great controversy. Enormous confusion prevailed as to whether the bacteria in this group could be considered, metabolically speaking, chemosynthetic, photosynthetic, neither or both.[56] By 1926, van Niel had found evidence, first, that they were actually photosynthetic (that is, they derived the energy for their metabolism from light) but that they still depended on the presence of hydrogen sulphide. Van Niel was even more excited when he found that some non-sulphur purple bacteria (*Athiorhodaceae*) "could develop in the same medium either anaerobically, but only if illuminated, or aerobically in complete darkness, so that for these organisms light and oxygen appeared to be equivalent".[57]

Despite the enthusiasm of his student, Kluyver could not be persuaded to accept this as the basis of a doctoral thesis. Instead, he encouraged van Niel to work on propionic acid bacteria, which were well known for their function in the ripening of certain types of Swiss cheese. Kluyver insisted that this would be a much better preparation for the work in industry that he anticipated for his students. Furthermore, Kluyver believed that, as purple bacteria grew so slowly, it would take van Niel too much time to get anywhere in his thesis. Van Niel reluctantly agreed to Kluvyer's suggestion, and in 1928 he received his PhD for a thesis on the biochemistry and morphology of propionic acid bacteria. Among other things, one of the questions that van Niel examined was the origin of the holes in Swiss Emmental cheese. Albeit amusing, this was definitely not the kind of fundamentally important microbiology in which van Niel had hoped to engage.

Yet by 1928, van Niel was fully convinced of the importance and justification of the line of research that Kluyver had initiated. Although microbiology was a science that had to deal with both practical and fundamental problems, van Niel believed (in agreement with Kluyver) that it was inappropriate to associate it primarily, as was usually done, with medical "bacteriology" or with the technical application of microbes in industrial fermentation, such as the brewing and dairy industries. Rather, he believed that microbiology should be conceived of as a branch of biology, and that it should be practised in a broadly encompassing and comparative way: general microbiology was what van Niel had in mind.[58] His vision had much in common with the programme pursued by general physiologists in the USA; it was also close to the gist of Otto Warburg's scientific approach as well as to the way (general) biochemistry was being practised by Frederick G. Hopkins at the University of Cambridge (UK), as I shall discuss later in this chapter. It was the search for fundamental communalities in the different realms of life that this generation of researchers shared. Van Niel

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[56] See van Niel (1941, pp. 264–269), for a review of the field up to van Niel's own work. Chemosynthetic bacteria are able to reduce carbon dioxide (or methane) in order to produce organic matter; while they use the oxidation of inorganic molecules (e.g. hydrogen gas, hydrogen sulfide) or methane as a source of energy, rather than sunlight.

[57] See van Niel (1967, p. 11).

[58] How he happened to develop this broad vision is explored in Chapter 1 of Spath (1999).

realised that he would be hard put to find an academic position in the Netherlands, where he could fulfil his vision. Thus, in 1928 van Niel and his family moved to California, where he had been offered a position at the Hopkins Marine Station in Pacific Grove, affiliated to Stanford University. In the Jacques Loeb Laboratory of this station van Niel would work for the next 35 years of his life.

At the time, experimental biology was being strongly promoted at Stanford University (as at many other institutions in the USA). It was a field that had started to thrive not the least because it was being fostered by the Rockefeller Foundation.[59] The establishment of the new laboratory at the marine station was part of this general development. Emulating, perhaps, the successful profile of the Zoological Station in Naples (Italy), where Otto Warburg once had learned about the latest trends of the field, the laboratory was not organised along disciplinary boundaries and thus brought together proponents of very different branches of biology, pursuing a number of different research themes. The young biophysicist Lourens G. M. Baas Becking was head of the laboratory; and while on sabbatical leave in the Netherlands in 1928, looking around for suitable staff to complete the marine station's profile, he succeeded in recruiting van Niel as an assistant professor. Although van Niel was happy to have found work at the marine station, he was nevertheless disappointed to discover that cooperation between the different scientists in residence did not work out quite as he had expected. This brought van Niel to develop a rather pragmatic attitude towards interdisciplinary cooperation:

> This experience taught me that the attack on a problem which requires the joint efforts of diverse specialists is likely to be successful only if it develops through the gradual accretion of a group whose members have already evinced a desire to work on specific aspects of that problem.[60]

Van Niel quickly settled down to working on purple bacteria, the growth of which he found to be greatly accelerated if they were continuously illuminated at high light intensities. He also continued thinking about the unity of metabolism—for example, the unity that existed in different types of photosynthesis. It is to van Niel's work on the photosynthesis of purple bacteria that I shall now turn.

4.3.2 Bacterial Photosynthesis and the Consequences

In 1929, van Niel presented, for the first time, the results of his six years of work on purple bacteria. This he did in form of a talk at a gathering of the Western Society

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[59] Opinions differ as to how to interpret this general shift to experimental biology. Kay (1993) suggests that there prevailed an agenda of "social control", exerted through a concerted campaign by the Rockefeller Foundation, a conservative American elite and some influential scientists. Spath (1999) points to the fact that, although the effects of this move were convergent, the interests and aims of individual scientists at the numerous institutions were very different.

[60] See van Niel (1967, p. 10).

of Naturalists, which that year was holding its traditional winter meeting in Pacific Grove. Van Niel's findings, as he later wrote:

> ... supported the view that photosynthesis can be considered as a light-dependent reaction in which different substances, specific for different kinds of photosynthetic organisms, serve as H-donors for the reduction of CO_2.[61]

This is an extremely dry formulation of what was then a completely revolutionary idea. In 1930 photosynthesis was still defined as a process in green plants (and algae) that produced oxygen—which *by definition* excluded the possibility that photosynthesis might occur without oxygen evolution.[62] Van Niel now set out to persuade people that the process familiar to plant scientists was only one out of a whole range of possibilities. This was going even further than the bold (and not very well received) suggestion that some years earlier had been proposed by a French plant physiologist, René Wurmser, reviving an earlier idea that had also been suggested by Georg Bredig (see Chapter 2), that the oxygen produced during photosynthesis might come from water.[63] In 1935 van Niel gave a succinct summary of his findings (given in the form of ten points):

> (i) There exist bacteria which can develop in entirely inorganic media containing H_2S, in the complete absence of oxygen, but only in the light.
> (ii) No development of these organisms takes place if H_2S is omitted.
> (iii) In media containing a sufficient quantity of $NaHCO_3$, ammonia-N (nitrogen in the form of ammonia), K, P, and Mg the amount of development is strictly proportional to the quantity of H_2S present.
> (iv) No development takes place in the absence of CO_2 (carbonate, bicarbonate).
> (v) Oxygen is not produced.
> (vi) During the development of these organisms H_2S becomes converted into S (green bacteria) or into H_2SO_4 (Thiorhodaceae).
> (vii) The reaction of the medium becomes more and more alkaline due to the disappearance of CO_2.
> (viii) Chemical analyses show that there exists a stoichiometrical relationship between the quantity of H_2S oxidised and the amount of CO_2 which has disappeared, to wit: for one molecule of H_2S oxidised to S, 0.5 molecule of CO_2 disappears (green bacteria); for 1 mol. of H_2S oxidised to H_2SO_4 almost 2 mol. of CO_2 (1.8) disappear.
> (ix) The carbon of the CO_2 which has disappeared can be recovered as organic carbon in the form of bacterial substance.
> (x) In the dark, in the absence of oxygen, no development takes place; H_2S is not converted into S or H_2SO_4, and there is no disappearance of CO_2.[64]

From these findings van Niel concluded that, if these bacteria really did convert carbon dioxide into an organic substance under the influence of light, one would be entirely justified to call this process "photosynthesis" and to describe the organisms

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[61] See van Niel (1967, p. 19).

[62] For a timeline of research in anoxygenic photosynthesis, see Gest and Blankenship (2004).

[63] See Wurmser (1921, 1926) and, especially, Wurmser (1930). Ideas on photosynthesis being a redox process were also expressed in Thunberg (1923), although he was still looking for an acceptable pathway to formaldehyde.

[64] van Niel (1935, pp. 138–139).

that carried out this type of metabolism as “photosynthetic”. The light-dependent process in the metabolic reactions of “photosynthetic bacteria”, in the sense described above, was formulated by van Niel as follows:

$$2\,CO_2 + H_2S + 2\,H_2O \xrightarrow[\text{light}]{} 2\,[CH_2O] + H_2SO_4$$

This was then compared with the usual formulation of the widely accepted summary equation for photosynthesis in green plants and algae:

$$CO_2 + H_2O \xrightarrow[\text{light}]{} [CH_2O] + O_2$$

Emphasising how strikingly these equations resembled each other, van Niel proposed the following general equation for photosynthesis:

$$CO_2 + H_2A \xrightarrow[\text{light}]{} [CH_2O] + 2\,A + H_2O$$

In this form, photosynthesis was understood to be the photochemically driven reduction of carbon dioxide with a variety of hydrogen donors. Of course, this concept also bore enormous consequences for the understanding of photosynthesis in plants and algae. Van Niel himself addressed this question in his 1935 paper:

> If one tries to understand the meaning of the generalized equation for photosynthesis it becomes clear that all those mechanisms proposed for the photosynthetic reaction which imply the formation of a carbonic acid-chlorophyll complex which is subsequently transformed into a formaldehyde peroxide are not quite in accordance with the formulation of photosynthesis as an oxidation–reduction process. Such schemes fail to give a satisfactory explanation for the photosynthetic processes carried out by the green and purple bacteria. From a unified point of view, as laid down in the generalized equation, green plant photosynthesis should be considered as a reduction of CO_2 with hydrogen obtained from H_2O, and the oxygen produced during illumination as dehydrogenated H_2O.[65]

This was a severe blow to the standard model. The weak point in van Niel’s argument, however, was that he was unable to propose a viable alternative—the generalised equation in itself was only a summary of the process, not a mechanism. As far as the photochemical part was concerned, van Niel was ready to follow Franck’s principal line of reasoning. The absorption of 4 quanta for the reduction of one molecule of carbon dioxide in green plants, van Niel argued, strongly suggested the activation of four water molecules in the photochemical reaction; and this activation was obviously brought about by the chlorophyll. As to the thermochemical part, which van Niel considered to be the reduction of carbon dioxide (and not, as was usually assumed, the removal of hydrogen peroxide!), he was convinced that some intermediate products had to exist, since all the redox reactions known then proceeded in small steps of one, or at most two, hydrogen atoms at a time; but what these products were remained an open question.[66]

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[65] van Niel (1935, pp. 142–143).

[66] van Niel (1935, p. 143).

As one might expect, researchers in photosynthesis were not yet ready to accept van Niel's new concept at face value. The idea that the molecular oxygen originates from water and not from carbon dioxide, still seemed outrageous to many scholars—although it had been suggested by various actors before that at least parts of the oxygen might come from the splitting of water; Bredig, Wurmser and Thunberg have already been mentioned in this book. It still would require an enormous cognitive leap (which many regarded as over the top) before scientists working in the field could drop this long-established assumption. The same held true for the other standard elements of photosynthesis models, such as the chlorophyll–carbon dioxide complex and the assumptions that the reduction of carbon dioxide was part of the light reaction, while the (dark) Blackman reaction was the removal of hydrogen peroxide. The suggestion that bacterial metabolism might be considered "photosynthetic" was equally preposterous to many of van Niel's fellow scientists. Yet, even though not many scientists were prepared to accept his suggestion in detail, van Niel's proposal provoked much discussion, and scientists around the world reacted to it—if only by attempting to provide a convincing rebuttal of this unthinkable possibility.

Far from considering his ideas to be revolutionary, van Niel himself believed, even in hindsight, that, given his background and his exposure to Kluyver's work, the suggestion that photosynthesis be investigated in the more general framework of hydrogen transfer reactions, was self-evident:

> It was a logical extension into the realm of photosynthesis of the general concept, then being developed by Kluyver and his co-workers, that fermentative as well as oxidative metabolic processes can be considered as composites of more or less elaborate series of consecutive and chemically intelligible step reactions, each one of which represented an inter- or intra-molecular transfer of hydrogen atoms from a donor to an acceptor molecule or site of a molecule. The results I had obtained by 1926 had shown that the purple sulfur bacteria, or *Thiorhodaceae*, can grow in strictly mineral media but only when exposed to light. This meant that they had to be considered as photosynthetic organisms. On the other hand, the requirement for H_2S and their failure to produce O_2 could now be interpreted to mean that they use H_2S as the specific H-donor for the reduction, or assimilation, of CO_2. The gist of this idea was incorporated by Kluyver & Donker in their epoch-making treatise on "Unity in Biochemistry".[67]

This surely does not do justice to the amount of conceptual work that van Niel must have undertaken to come up with his general equation (it was, for example, by no means self-evident that an organism that requires light for growth should be considered "photosynthetic"). However, the exposure for some years to Kluyver's general theory that metabolic reactions occur by hydrogen transfer is very likely to have promoted the generation of these ideas. The parallel between van Niel's concept and Kluyver's programme was already clear at the time: comparative microbiology was the theme of van Niel's first seminar at the Hopkins Marine Station. As early as his lecture of 1929, six years before he published his concept of the general photosynthesis equation, van Niel had laid great emphasis on the fact that the study of the metabolic pathways of such inconspicuous organisms as *Thiorhodaceae* could

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[67] van Niel (1967, p. 10).

contribute to a better understanding of the metabolism of a wide range of higher organisms. He wrote:

> And here especially lies the importance of the study of these "abnormal" photosynthetic processes, because a comparison of the factors and conditions which are required for their accomplishment will enable us to find those characteristics which are common to all. It will then be possible to derive the fundamental laws underlying all photosynthetic processes and to correlate these into a general view.[68]

Van Niel's own achievements were the most compelling evidence for this sweeping statement. He provided a new and utterly unexpected link between general microbiology, general physiology and general biochemistry. His amenable personality made this link appear even more compelling and inspired many young researchers to study bacterial photosynthesis—a research theme that did not exist before 1929. Van Niel's summer courses in General Microbiology quickly became internationally renowned, and after a few years the Hopkins Marine Station had turned into a thriving research centre for this discipline. In the next section, I shall take a look at the work of a contemporary scientist, who at first strongly opposed van Niel, but eventually became one of the latter's staunchest supporters: the aforementioned Hans Gaffron, a German chemist-turned-microbiologist, who, together with Kurt Wohl, developed in 1936 the bold hypothesis of a functional and physical "photosynthetic unit" in plants.

4.4 The Photosynthetic Unit

Hans Gaffron spent the first ten years of his life in Lima, Peru, where his father Eduard had settled as an affluent physician.[69] In 1912, after his father retired, the family returned to Germany, and in 1920 Gaffron started his studies in chemistry at the universities of Heidelberg and Berlin. His academic career began in 1925, with a doctoral thesis completed at the Chemical Institute of Berlin's Friedrich Wilhelm University under the supervision of Wilhelm Traube. In the same year, Gaffron was appointed to the post of Research Assistant in Otto Warburg's department at the Kaiser Wilhelm Institute (KWI) for Biology. He was thus working with Warburg when Robert Emerson arrived to take up his doctoral studies; and a friendship between Gaffron and Emerson developed that was to last for the rest of their lives. Like Emerson, Gaffron became thoroughly familiar with the technique of manometry, which also continued to be his preferred measuring method after leaving Berlin.

Gaffron stayed with Warburg for six years, an unusually long period for a research scholar in this laboratory, which testifies to the good working relationship they must

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[68] van Niel (1930, p. 168). Quoted in Spath (1999, p. 117).

[69] The biographical information on Gaffron was taken from Rürup (2008, pp. 199–201). For a tribute to Gaffron and his co-workers, with special emphasis on Gaffron's work on the hydrogen metabolism in green algae, see Homann (2002).

have had. In 1931, Gaffron went to Caltech in the USA for a year as a guest, where he worked in close proximity to Emerson and Arnold, who were carrying out the crucial flashing light experiments for their 1932 paper. Gaffron then spent some time at the Zoological Station in Naples (Italy), before moving back to Berlin, in 1933, to accept a position as a Research Assistant at the KWI for Biochemistry. In 1936, when the institute's Jewish director Carl Neuberg was forced to take early retirement, all his employees, including Gaffron, were dismissed. Although Gaffron was able to find a temporary position in Friedrich von Wettstein's department at the KWI for Biology for the next year and a half, his prospects looked bleak, particularly given the fact that, according to his family, he not only opposed the Nazi Government but also sympathised with the Communists.[70] Thus, at the end of 1937, Gaffron and his wife left for the USA and took refuge in the laboratory of van Niel (see below) at the Hopkins Marine Station in California. It seems that Otto Warburg was unusually supportive in organising Gaffron's emigration: Gaffron himself believed that he would not have settled down so easily, had it not been for a letter that Warburg wrote to the officials of the Rockefeller Foundation, which contained a "magic spell" that opened up doors for Gaffron—and provided him with a Rockefeller Fellowship for the first 6 months of his stay at the marine station.[71] Later, in the autumn of 1939, Gaffron was invited by James Franck to become his research associate at the University of Chicago; Gaffron would remain affiliated to this institution for the next 20 years of his life.

Bacterial photosynthesis was to become Gaffron's main research theme, although he is perhaps best known for his discovery of hydrogen metabolism in green algae, which had decisive consequences for conceptualising photosynthesis at large. Gaffron first moved into studies on bacteria in 1933 with emphasis on the metabolism of non-sulphur purple bacteria (*Athiorhodaceae*). His achievements here were seminal in their own right, even if Gaffron later had to revise some of his interpretations. In 1933 Gaffron explained his interest in this bacterial group with the aim of adding yet another variant of photosynthesis to the alternatives that had been known up to then (chlorophyllous photosynthesis and van Niel's study of *Thiorhodaceae*).[72] The interpretation of some of his data on the metabolism of purple bacteria sparked off much contention between Gaffron and van Niel, in particular on the question as to whether or not organic substances could serve as hydrogen donors in purple sulphur bacteria. In 1931, van Niel had purported that this was the case, while Gaffron

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[70] Warburg was allegedly instrumental in securing Gaffron this position in the KWI for Biology through his good connections with Friedrich Glum, Administrative Director of the Kaiser Wilhelm Society at the time; cf. Werner (1988, p. 246).

[71] Werner (1988, p. 246); Rürup (2008, p. 201).

[72] Gaffron (1933b, p. 2). This general aim during these years was shared by Charles Stacy French, another giant in twentieth century photosynthesis research. In French (1937, p. 71), the latter wrote: "It is with the hope of finding a new approach to green plant photosynthesis that several workers are now studying the different kinds of photoassimilation in these bacteria. Probably by defining the differences between green plants and purple bacteria CO_2 assimilation, the chemical mechanism of both will become clearer".

claimed, in 1934, to have found evidence to the contrary. This was countered by van Niel, one year later, with the conjecture that Gaffron had used cultures that were contaminated—which understandably infuriated Gaffron.[73] The argument was only settled after van Niel carried out joint experiments with Gaffron at Warburg's laboratory in Berlin. Neither of them could ever have imagined that, only a short while later, Gaffron would obtain a research position in van Niel's laboratory at the Hopkins Marine Station in California.[74]

It was also in these years that Gaffron became intrigued by the role of molecular hydrogen in bacterial metabolism. In 1934, the Dutch microbiologist Pieter Roelofsen, who was working in Utrecht, the Netherlands, claimed that molecular hydrogen was able to support the photosynthetic reduction of carbon dioxide by sulphur bacteria.[75] Gaffron checked this out with his own strains of bacteria and was able to fully confirm the finding. Hydrogenase, the enzyme responsible for the oxidation of hydrogen, and its role in photosynthesis became henceforth one of his main research themes.

4.4.1 Context and Scope of the 1936 Paper

The contribution to photosynthesis research for which Gaffron's name is most vividly remembered is the paper that he co-authored with the German physicist Kurt Wohl in 1936.[76] In it, the two young men set out to argue why the standard model of photosynthesis was no longer tenable. (It is important to note that, at the time, Gaffron had not yet accepted van Niel's generalised equation for photosynthesis). In view of the results of the 1932 Emerson–Arnold experiments described earlier, Gaffron and Wohl maintained that one had to drop the idea, once and for all, that every carbon dioxide molecule was assigned to one specific molecule of chlorophyll. Rather, the energy absorbed by a large number of chlorophyll molecules could be made available, in an unlocalised sense, to a single carbon dioxide molecule. Hence, chlorophyll did not act as a "photoferment" in the reaction but as a sensitiser.

These last two proposals had already been made three years earlier by Gaffron.[77] However, Gaffron and Wohl acknowledged in their introduction that it was only thanks to the impact of the discussions that took place at a seminar organised by the German physicist Max Delbrück in Berlin that these ideas had been elaborated and used as the basis for a new concept of photosynthesis.[78] This seminar was the very discussion circle that had produced, in 1935, the celebrated landmark paper

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[73] van Niel (1931); Gaffron (1934); van Niel (1935).

[74] See Homann (2002, p. 94). Gaffron (1963) provides an account of his dispute with van Niel.

[75] See Roelofsen (1934).

[76] Gaffron and Wohl (1936).

[77] Gaffron (1933a).

[78] See Gaffron and Wohl (1936, p. 81).

"On the Nature of Gene Mutation and Gene Structure", co-authored by Delbrück, the geneticist Nikolay W. Timofeev-Ressovsky and the physicist Karl G. Zimmer.[79] The paper is also known as the "Three Man Paper" (or 3MP; originally German: *Dreimännerwerk*, *Dreimännerarbeit*) and is often regarded as the stimulus behind what would later become the discipline of molecular biology.[80] Thus, the whole context is worth a little further consideration.[81]

Delbrück, who was originally trained as a physicist, had gone to Berlin in 1932 to work at the KWI for Physical Chemistry as an assistant to Lise Meitner (mentioned earlier as Franck's close friend and colleague). Before that, Delbrück had spent some months in Copenhagen with Niels Bohr, who was then elaborating on the deeper meaning of quantum mechanics—in particular, the complementarity principle. Later Delbrück recalled that Bohr had vigorously spoken of the possibility that this new quantum mechanical dialectic might also be relevant to other areas of science, such as how "life" was related to physics and chemistry. It was through these discussions that Delbrück became acquainted with (and fascinated by) current problems in biology and their potential reinterpretation from the viewpoint of physics.[82] In Berlin, Delbrück decided to explore, together with like-minded scientists, the potential application of current knowledge and expertise in physics to biological phenomena. In an interview of 1978, Delbrück recalled how he had invited a group of five or six theoretical physicists to join him for informal discussions at his family home in 1934. The group met at irregular intervals, sometimes weekly, sometimes once a month, until Delbrück left Germany in the summer of 1937 (that is, shortly after Gaffron had left for the USA). This is how Delbrück described the early phase of this discussion circle:

> This little club which started out as theoretical physics, and then brought in genetics, also brought in biochemists and photosynthesis physiologists. The photosynthesis man was Hans Gaffron, and he and Kurt Wohl lived together [with their families] in the same house in Dahlem [a district of Berlin]. As a result of the talks that we had in our club on photosynthesis, they published a series of papers on the kinetics of photosynthesis. [. . .] There were some more sophisticated experiments on this kinetics that had been published. Wohl and Gaffron discussed these experiments, and essentially already described what is now accepted; namely, that photosynthesis is done in photosynthetic units, which consist of about 1000 molecules of chlorophyll all funneling their energy into one photosynthetic reaction center.[83]

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[79] Timofeeff-Ressovsky et al. (1935).

[80] It is not entirely clear how this name relates to the other famous "Three Men Paper", likewise known in German as *Dreimännerarbeit*: the paper by Max Born, Werner Heisenberg and Pascual Jordan of 1926 in which they introduced the matrix mechanics formulation of quantum mechanics; see Born et al. (1926).

[81] For more detailed information on the Delbrück seminar and the development of biophysics in Berlin at the time, see Sloan (2009).

[82] See the interview with Delbrück, carried out as part of the Caltech Archives Oral History Project, for the latter's recollections of these years; Delbrück (1978, in particular p. 41).

[83] Delbrück (1978, p. 55).

Thus, even so many years later, Delbrück still vividly remembered that the discussions had centred on photosynthesis. Delbrück had been interested in the photochemical reactions in biology long before he began to dwell on the gene and its structure. Genetics was really only one of the themes that kept the group together. In addition to Gaffron, two other "photosynthesis physiologists" are reported to have participated in Delbrück's circle, at least occasionally. The American plant physiologist Charles Stacy French wrote in his autobiography that, when he went as a postdoctoral student to Otto Warburg's laboratory in Berlin in 1935, he did not see much of the famous Dahlem science institutions outside the laboratory, "except for a few seminars on photosynthesis at Max Delbrück's house with Hans Gaffron and Eugene Rabinowitch".[84] Although these may have been private discussions that were not part of the official Delbrück seminars, it may not be far-fetched to consider them within the same context. Thus, French and Rabinowitch should probably be included in the list of discussants.

Rather than a series of publications, as Delbrück recalled, the main output of these discussions was a single common paper of 1936, published in two parts. This was followed, four years later, by a comprehensive review of the same problem in English by Wohl.[85] The principal argument of the Gaffron–Wohl paper was very similar to the thrust of the Three Man Paper on the mutation and structure of the gene: Data obtained in biological systems were systematically and carefully subjected to an interpretation from a quantum physics point of view.

4.4.2 *Critique of the Standard Model*

Gaffron and Wohl began their paper with an outright rejection of the standard model of photosynthesis, which was described earlier in this chapter. They first turned to Franck's notion of the photochemical process, which involved four reactions, each of which was initiated by one light quantum. This, according to Franck, eventually led to the formation of formaldehyde and hydrogen peroxide, according to the following equation:

$$CO_2 + 3\,H_2O \xrightarrow[\text{light}]{} CH_2O\ \text{(formaldehyde)} + 2\,H_2O_2$$

Gaffron and Wohl pointed out that this pathway was impossible, since the reduction of carbon dioxide to formaldehyde required more energy than the 4 red light quanta could provide, which were found sufficient for photosynthesis to take place. The energetic account would look more promising, if one assumed that, instead of free formaldehyde, a carbon moiety developed at the same oxidation stage, which then

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[84] French (1979, p. 7). On the life and work of French, see Govindjee and Fork (2006).

[85] Gaffron and Wohl (1936); Wohl (1940). Being of Jewish origin, also Wohl had to leave Germany in 1933 and emigrated to Oxford, England, where he was able to find a position at Balliol college; see Jost (1963).

remained bound to other components. Yet, even if this were the case, there were other difficulties: *first*, each of the photochemical steps had to run very efficiently; *second*, each of them had to have a very small activation energy threshold (since only about 40 kilocalories (kcal) were available to initiate the four reaction steps, in addition to, as was generally assumed, forming a peroxide); and *third*, the intermediary photoproducts had to have a very long lifespan (of, at least, some seconds) before the next light quantum arrived. "Unfortunately, one usually has to choose between these three desired properties: high yield, low activation energy, long lifespan", Gaffron and Wohl pointedly concluded.[86] Back reactions and unstable intermediates were only some of even more problems, which the H/OH exchange mechanisms suggested by Franck implied.

The second major problem of the standard model was that chlorophyll was thought to be an actual reactant. This was at variance with the fact that, at least in vitro, chlorophyll had been found to be rather inert, particularly in terms of photochemical reactions. And, although Stoll had suggested that in the living cell chlorophyll would be more reactive (since it was in a colloidal state and bound to specific cell proteins), Gaffron and Wohl argued that Stoll had been unable to produce any evidence to support this claim. They also found it highly improbable that magnesium should be the site where carbon dioxide was bound, as the standard model suggested (following Stoll and Willstätter). No measurable change in the chlorophyll's absorption spectrum had ever been detected during photosynthesis, although this is what one would have expected if magnesium were the binding site.

4.4.3 The Unit as Explanatory Alternative

The authors then turned to the experimental evidence that had been left unexplained by the standard model. First, the well-known finding of Warburg and Negelein (1923) that photosynthesis needed only 4 light quanta to reduce one molecule of carbon dioxide and to release one molecule of oxygen. Gaffron and Wohl set out to check whether, in these classic experiments, every single chlorophyll molecule did actually receive 4 light quanta, as set out in the standard model. Under the conditions chosen by Warburg and Negelein, the result was clearly negative: "In this experiment only 0.8 % of the chlorophyll molecules could have received the four light quanta necessary to reduce 'their' molecule of carbonic acid".[87] The key to the solution of this puzzle, Gaffron and Wohl maintained, was provided by the experiments carried out in 1932 by Emerson and Arnold, who had found that, at maximum efficiency, one molecule of carbon dioxide was reduced per (roughly) every 2500 molecules of chlorophyll, which, therefore, they called "one unit".

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[86] Gaffron and Wohl (1936, p. 82).

[87] Gaffron and Wohl (1936, p. 86).

Gaffron and Wohl suggested that this had to be taken very seriously: “This means that a molecule of carbonic acid will be reduced as soon as four [light] quanta are absorbed by any of the chlorophyll molecules within this unit”.[88] This could not possibly be reconciled with the Stoll–Franck theory, they argued, but it was able to explain the excellent quantum yield in Warburg and Negelein’s experiments. Further confirmation was provided from Emerson and Arnold’s estimation of the Blackman reaction’s time span, which was no more than 0.02 seconds: “This means that, according to our earlier considerations, every molecule of carbonic acid that is bound in a state in which it is susceptible to [photosynthetic] assimilation [. . .] must receive in these 0.02 seconds, at the given stationary [light] intensity, four light quanta”.[89] Under the given experimental conditions, this was only possible if approximately 1000 molecules of chlorophyll acted together, which Gaffron and Wohl considered the number of *active* chlorophyll molecules that formed one photosynthetic unit.

The mechanism underlying this cooperative action of the pigments was, however, far from clear. Gaffron and Wohl considered two possibilities. Either the carbonic acid and its photochemically produced derivatives were not fixed but moved in a continuous diffusion from one chlorophyll molecule to the other, picking up energy quanta on the way. Or, alternatively, the carbonic acid was bound to one determined location, where it was reduced, while the energy that had been absorbed within the assimilatory unit moved around very quickly until it passed the site of reduction and was used up. Gaffron and Wohl favoured the second option, and speculated that the carbonic acid might be bound to one of chlorophyll’s nitrogen atoms (although they had to admit that there was no conclusive evidence for this hypothesis). In any event, Gaffron and Wohl emphasised that, in view of the fact that for the whole of photosynthesis only 4 light quanta were required, the involvement of high-energy intermediates, such as peroxides, was out of the question. Yet, Gaffron and Wohl, unfortunately, could offer no convincing alternative to the underlying chemical pathway.

4.5 Franck’s Conservative Alternatives

4.5.1 Franck and Herzfeld’s Proposal

The critical objections raised by Gaffron and Wohl against Franck’s photosynthesis model of 1935 were, in principle, accepted by Franck, particularly the objection that the intermediate products would have to be too long-lived to provide a realistic option. In his subsequent papers Franck himself added a number of further points of critique, and by 1937, he had developed a new proposal, together with the physicist Karl F. Herzfeld. They contested the view that recent experiments “make the assumption of

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[88] Gaffron and Wohl (1936, p. 87).

[89] Gaffron and Wohl (1936, p. 88).

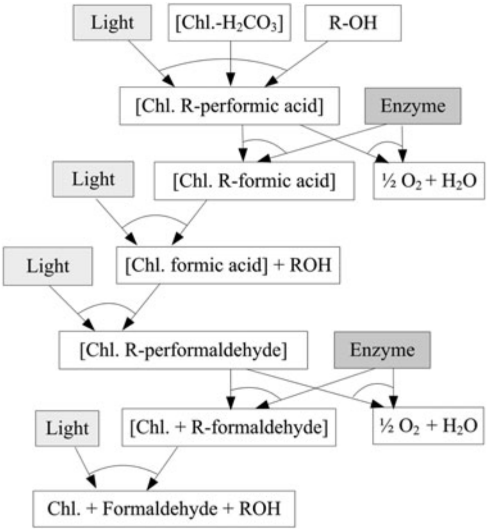

Fig. 4.5 The model of Franck and Herzfeld (1937).

a photosynthetic unit necessary, in which a large number of molecules cooperate in a way not encountered *in vitro*".[90] Their strongest argument against the existence of a photosynthetic unit (which most researchers in photosynthesis shared) was the difficulty involved in imagining a model of the underlying mechanism that would allow for the effective transfer of energy in the chlorophyll from the site of absorption to the site of carbonic acid reduction.

Franck and Herzfeld started by considering the fact that leaves emit fluorescence, which seemed to indicate that the chlorophyll in vivo was not in an aggregated but in a unimolecular state. This, the authors thought, could not be reconciled with the concept of a photosynthetic unit. In their alternative model, Franck and Herzfeld tried to accommodate this finding, as well as the assumptions that no more than 4 quanta were necessary for the process (which for most people at the time was taken to indicate the existence of four photochemical steps) and that chlorophyll formed a complex with carbonic acid. The pathway they suggested went through the stages of a peroxy acid, formic acid and a peroxy aldehyde. These, as Franck and Herzfeld did not fail to mention, "are the same intermediate compounds as in auto-oxydation processes, so that the similarity between these two inverse processes is striking". The unexpectedly low light saturation point measured by Emerson and Arnold was explained "by back chain reactions initiated by photolytical decomposition of the per-compounds".[91]

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[90] Franck and Herzfeld (1937, p. 238).

[91] Franck and Herzfeld (1937, p. 237).

Furthermore, Franck and Herzfeld believed that the chlorophyll–carbonic acid complex was bound to an organic molecule, which they called ROH: “The ROH may for instance be a protein which forms the main body of the chloroplasts, on the surface of which the chlorophyll is adsorbed. The chlorophyll molecules will then be able to move along the surface as a two-dimensional gas”.[92] This idea was introduced in order to avoid the notion of the existence of free radicals, which appeared to be incompatible with the high quantum yield of the process. Yet, unlike Gaffron and Wohl, Franck and Herzfeld still supported the assumption that formaldehyde was the first reduction product, in the course of which “probably two peroxide molecules are formed, which, under the action of an enzyme, split off oxygen”.[93]

The mechanism is reconstructed in a graph form in Fig. 4.2. Four light reactions corresponded to the 4 light quanta that were required for the process, while the two enzyme reactions constituted the temperature-dependent part, that is, the Blackman reaction. Franck and Herzfeld defended Willstätter’s assumption that the photochemical reaction steps consisted of an exchange of hydrogen versus hydroxyl groups in the carbonic acid molecule; however, they tried to divide these exchange reactions into four energetically reasonable single quantum reactions. The first photochemical reaction consisted of the formation of performic acid from carbonic acid and the ROH in a complex loosely bound to chlorophyll; the third was the formation of performaldehyde from formic acid, in which again the ROH was involved. Both these reactions were “followed by dark reactions in which the peracid or the peraldehyde is reduced under the influence of enzymes to the acid and the aldehyde”, which restored the ROH.[94]

There remained, however, the uncomfortable observation made by Emerson and Arnold that the maximum assimilation rate was much lower than one would expect if every chlorophyll molecule worked as an autonomous entity. Franck and Herzfeld suggested that the photochemical products, which normally released the oxygen, would at high light intensities be frequently hit by further light quanta and, consequently, disintegrate, thereby initiating chain reactions which destroyed other photoproducts and, hence, reduced the process’s overall efficiency. Both authors considered the concept of a hypothetical photosynthetic unit (in other words, a hitherto unheard-of photochemical process) to be unacceptable.

While Franck and Herzfeld’s proposal was the most influential alternative to the concept of a photosynthetic unit, other explanations of the low oxygen yield at the light saturation point were also being offered. Emerson himself, for example, considered it likely that the rate of photosynthesis in flashing light of high intensity was limited by an essential molecule, which was present in an amount of only

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[92] Franck and Herzfeld (1937, p. 240).

[93] Franck and Herzfeld (1937, p. 239).

[94] Franck and Herzfeld (1937, p. 240).

about 1/2500 of the chlorophyll.[95] Optical models, which tried to fill in the gaps of the "unit" concept, were also proposed, beginning with two papers by Wohl.[96] In these models it was assumed that there was a rapid transfer of absorbed energy from excited chlorophyll molecules to specific reaction sites (of unknown material nature and one per several thousand molecules of chlorophyll) to which the carbon dioxide was attached. However, none of these options was unreservedly accepted in the community. The general state of the discussion, therefore, was succinctly summarised in 1938 by the plant physiologist Winston Manning:

> The existence of a photosynthetic unit has thus far been neither proved nor disproved. Its existence would offer an explanation for several different groups of experiments, but on the other hand, various arguments largely based on physical grounds, can be offered against it.[97]

4.5.2 Franck's Further Attempts

In the years that followed, Franck used a number of further approaches to reaffirm his criticisms of the concept of a photosynthetic unit. One of these was a paper on the migration of the excitation energy in crystals, which he co-authored in 1938 with Edward Teller, a nuclear physicist of Hungarian origin, who would later find fame as the "father of the hydrogen bomb".[98] Although at first glance, the paper seems to have nothing to do with photosynthesis, on closer inspection one realises that Gaffron and Wohl's explanation of the energy transfer in a photosynthetic unit directly stimulated the work.[99] Gaffron and Wohl had considered the possibility that the chlorophyll molecules of a photosynthetic unit might be organised in the form of a one-dimensional crystal to which carbon dioxide molecules were attached, one at each end.[100] Energy absorbed at any point of this crystal would then migrate through the crystal and be channelled towards the carbon dioxide molecules. Franck and Teller maintained that this was highly improbable, because the migration of

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[95] Emerson (1936). In his obituary of Emerson in 1961, Rabinowitch considered this to have been the most generally accepted interpretation. See Rabinowitch (1961, pp. 118–119). Emerson also used the terms "catalyst" or "photoenzyme". The concept aligns neatly with today's conception of "reaction centres".

[96] Wohl (1940, 1941).

[97] Manning (1938, p. 156).

[98] On Teller see, e.g., Rhodes (1995); Teller (2001).

[99] Franck and Teller (1938, p. 861).

[100] Again, it is striking how closely this resembles the idea, which was elaborated in the Three Man Paper, of the gene being similar to a crystal; cf. Timofeeff-Ressovsky et al. (1935).

excitation energy would be bound to trigger a much higher level of fluorescence in photosynthesising leaves than was actually the case.[101]

By 1941, Franck and Herzfeld presented yet another model—not because of inherent weaknesses of the 1937 version, the authors explained, but because of decisive new developments in the field which had made most of their earlier work obsolete.[102] Most importantly, by the end of the 1930s, a number of photosynthesis researchers working in the USA had started to doubt the validity of Warburg and Negelein's proposal that 4 light quanta were the minimum requirement for photosynthesis. A value of 10–12 light quanta seemed to be more realistic, and this boost of the energy budget (by the factor 2–3!) radically changed and, in fact, greatly alleviated, the task of modelling the process (the ensuing controversy is discussed in chapter 5). Furthermore, Samuel Ruben, Martin Kamen and their co-workers in Berkeley had found, with the help of radioactive carbon isotopes, that in photosynthesis carbon dioxide reacted with an acceptor molecule, RH, in a carboxylation process, the result of which was the formation of R–COOH (see Chapter 6 for a discussion of Ruben and Kamen's work). And, finally, Franck and Herzfeld cited work on the chlorophyll fluorescence of photosynthesis, partly carried out by Franck himself. They had found, for example, that the addition or removal of carbon dioxide produced changed the rate of chlorophyll fluorescence in proportion to the rate of photosynthesis. Franck and Herzfeld saw this as a strong indication of the fact that carbon dioxide was in direct energy exchange with the chlorophyll molecules: "Theories which assume that the photochemical part of photosynthesis results merely in a production of some reducing substance, which in turn reduces carbon dioxide in a mechanism chemically independent and spatially separated from the chlorophyll, are not in accordance with these observations".[103]

Franck and Herzfeld were still convinced that the number of light quanta required corresponded closely to the number of photochemical steps involved and, hence, the number of intermediates produced. In view of the new quantum yield value, they estimated that the number of steps had to be eight, in order to allow for some inefficient absorbance. The carboxylation reaction identified in Berkeley was cyanide sensitive, which, Franck and Herzfeld believed, demonstrated that it was promoted by a catalyst, which they called *A*. Franck and Herzfeld considered the product of this reaction, R–COOH, to be the substance that underwent further photochemical changes.

Molecules of the type R'H were thought to act as hydrogen donors, while the energy required for the transfer of hydrogen was supplied by the light energy that the chlorophyll had absorbed. The reduction of one molecule of carbon dioxide required the transfer of four hydrogen atoms, while the four remaining R' radicals regained

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[101] On the paper's argument, see also the review by Franck and Gaffron (1941, p. 210). However, Franck and Teller assumed that there existed a one-dimensional structure (a linear chain of chlorophyll molecules); the application of two- or three-dimensional models, in, e.g., Bay and Pearlstein (1963), led to very different results. See Pearlstein (2002) for a short review.

[102] See Franck and Herzfeld (1941).

[103] Franck and Herzfeld (1941, p. 979).

their hydrogen by oxidising water molecules. Again, the energy had to be supplied by four additional instances of absorption. In view of the fluorescence experiments cited earlier, Franck and Herzfeld assumed that the carbon dioxide reduction was directly connected to the photochemical steps, so that the hydrogen transfers were produced by the chlorophyll's excitation energy. This implied, they believed, that the chlorophyll molecule took part in these reactions:

> R'H molecules then have to be members of the molecular complex containing the chlorophyll molecule itself and RCOOH or its derivatives. It simplifies the picture if one identifies the R'H molecules with the chlorophyll itself. In other words, one adopts the often-discussed idea that the chlorophyll not only acts as a sensitizer but also undergoes chemical reactions during photosynthesis. Indeed, the results of some new experiments with chlorophyll in organic solution make that hypothesis very probable.[104]

In order to explain the light saturation curve of photosynthesis as well as the flashing light experiments of Emerson and Arnold, the authors introduced what they described as a "very simple hypothesis":

> The limiting dark reaction is a process in which catalyst molecules present in a concentration several thousand times smaller than the concentration of chlorophyll operate on a photochemical product which is chemically very unstable. The catalytic reaction stabilizes the photoproduct. All the photoproducts not stabilized during their lifetime are eliminated by back reactions.[105]

The catalyst responsible for stabilising the reaction was called B. Franck and Herzfeld believed that each catalyst B molecule stabilised only one molecule of photoproduct, while all the others would be subject to back reactions. If the time interval between two flashes were greater than B's recovery period, on the arrival of the next wave of photoproducts all the B molecules would be available and, hence, the efficiency of the process would be at its maximum. Furthermore, Franck and Herzfeld thought that, since all the photochemical steps were so similar—they were shifts of hydrogen atoms from one bond to another—catalyst B would stabilise the products of *all* the photochemical steps. They formulated the reaction sequence as follows:

$$\text{Chl.-RCOOH} \underset{\text{light}}{\longrightarrow} \text{Chl.'}_1$$
$$\text{Chl.'}_1 + \text{Catalyst B} \longrightarrow \text{Chl.*}_1 + \text{Catalyst B}$$

Chl.*_1 would then undergo the next photochemical step, the intermediate product of which (Chl.'_2) would be converted into Chl.*_2, and so forth. The chlorophyll would be replenished with hydrogen again through the formation of peroxide radicals, which had to be removed by the action of a third catalyst, C. The rest of the paper then focused on a detailed analysis of the differential equations that were supposed to demonstrate the model's validity—although one can safely assume that Franck's more biologically oriented colleagues were hardly able to appreciate them. They were far more interested in getting to know more about biochemical work that was

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[104] Franck and Herzfeld (1941, p. 982).

[105] Franck and Herzfeld (1941, p. 985).

being done, at the same time, in Cambridge, UK, which will be introduced in the following section.

4.6 Isolated Chloroplasts and Water Splitting

4.6.1 Robert (Robin) Hill and the Chloroplast Reaction

The biochemical work done by Robert Hill[106]—or "Robin" as he was usually called by friends and colleagues—began at the Cambridge School of Biochemistry of the University of Cambridge (UK), which was strongly dominated by Frederick G. Hopkins's vision of general biochemistry.[107] At the time biochemistry was, for the most part, restricted to the study of animal and human metabolism, and the approach adopted by the Cambridge department (together with a few other British institutions, such as the groups headed by Sir Rudolph Peters at the University of Oxford and by David Keilin at the Molteno Institute, Cambridge) was a notable exception.[108] The general biochemistry practised at these places covered a broad range of fundamental biological topics, such as growth, development, nutrition and energy transformation, which were then studied within all forms of life: bacteria as well as animals, plants as well as invertebrates. Already in his celebrated 1913 lecture to the British Association for the Advancement of Science (BAAS), Hopkins had underlined that all forms of life were unified at the metabolic level and that this unity had to be represented in the way metabolic processes were studied.[109] Hopkins even used this as an argument for introducing the study of biochemistry as an independent discipline (which, at the time, frequently met with the objection that it was too narrow a field of study).

When Hill arrived (in 1919) as an undergraduate student at the University of Cambridge to specialise in chemistry (although he was also deeply interested in plants), Hopkins's department was on the point of entering a period of enormous expansion.[110] While in 1920 the Hopkins group counted ten workers, by 1925 this number had soared to 59.[111] Hill had already demonstrated an extremely broad range of talents, being "equally master of plant morphology, physiology, and organic and physical chemistry"; he was also described by his contemporaries as "the shy genius

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[106] A special 1992 issue of *Photosynthesis Research* was dedicated to the memory of Hill; see Rich (1992). See Bendall (1994) for a biographical account and Walker (2002) for a tribute to Hill's work on chloroplasts. Hill (1965) provides an autobiographical perspective.

[107] On Hopkins and his institute, see, e.g., Needham et al. (1949) as well as Kohler (1982); Chapter 4.

[108] On the history of biochemistry in the early twentieth century, see Holmes (1986).

[109] See Hopkins (1949). He argued along similar lines in Hopkins (1926).

[110] Hill was admitted as a scholar to Emmanuel College in 1917; however, he only started seriously reading the Natural Sciences Tripos after the end of the First World War. Bendall (1994, pp. 145–146).

[111] See Kohler (1982, p. 81).

type".[112] Originally, Hill had intended to embark on a serious, biochemical study of natural dyes and plant pigments, a subject that had engrossed him for some time already.[113] However, Hopkins was less than enthusiastic about this research theme and advised Hill to work on haemoglobin instead. Hill did as directed, and from 1925 he produced a series of fine papers on this subject.

In 1924, Hopkins's Biochemical Laboratory moved into its new (and now famous) building on Tennis Court Road, thereby coming into the immediate vicinity of the Molteno Institute, where, in the years 1920–1925, Keilin carried out his seminal studies of cytochromes.[114] On the occasion of a public presentation of plant pigment solutions, for which he had prepared a number of specimens, Hill met Keilin, and was invited by the latter to join him in his work on cytochromes. Hill happily accepted and became a regular (if not daily) visitor to the Molteno Institute; he spent a full year trying, by all the means available, to isolate cytochrome *c*.[115] Hill continued to work with Keilin until the latter's death in 1963; and their collaborative effort exerted an enormous influence on the rest of Hill's career. It was while researching into cytochromes and related compounds, under the supervision of Keilin, that Hill learned the spectroscopic methods that he would later utilise to measure the activity of isolated chloroplasts. And it was Hill's thorough knowledge of the chemistry and biophysics of cytochromes, acquired in Keilin's laboratory, that led him to propose (in 1960 with Fay Bendall) the mechanism that would later become known as the "Z-scheme" of photosynthesis (see Chapter 7).

Keilin came to his research into cytochromes somehow accidentally through his investigations of peculiar haemoglobin phenomena in the larva of a horse parasite (it turned out that this bug was able to store oxyhaemoglobin for emergency use under anaerobic conditions). This noteworthy case led Keilin to study cellular oxidation; and he rediscovered a compound that had already been described in 1886 by the English physician Charles A. MacMunn. Keilin gave it the name "cytochrome" (which is the Greek for "cellular pigment"). In a celebrated paper of 1925, Keilin argued that this compound was "one of the most widely distributed respiratory pigments" in existence.[116] Keilin was able to characterise this pigment by its unique absorption spectrum of four bands, which he found uniformly present in many different forms of life. He also noted that the property of being reversibly oxidised seemed to be a characteristic of the compound. This first communication was complemented

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[112] Kohler (1982, p. 83).

[113] Hill never lost this interest in plant pigments, and he became a well-known expert in the chemistry of natural dyes. Hill invariably grew the material for these and other studies in his own garden. He was also very skilled in extracting pigments and used them, among other things, for his own watercolour paintings. See Bendall (1994, p. 143).

[114] Keilin was made director of the institute in 1931. On Keilin's life and work, see Mann (1964). Keilin started his career with a strong interest in beetles and became a proficient entomologist. Even during the years of his research into cytochromes, Keilin never gave up his pursuit of questions on the morphology and physiology of insects.

[115] See the papers by Keilin et al. (1931) and Keilin and Hill (1933).

[116] Keilin (1925b, p. 315).

by a second (and equally celebrated) paper later in the same year in which Keilin made his first suggestions concerning the cytochrome's active function in cellular oxidations and reductions.[117] This short sketch of Keilin's work shows that he shared several points of common interest with Hill: both had worked on haemoglobin and its oxidised state; both became skilled in using spectroscopic methods; and both had organismic and biochemical interests. In his further studies in cellular respiration, Keilin was instrumental in conceptualising the respiratory electron transport chain; Hill, who was clearly inspired by Keilin, would later model photosynthesis along very similar lines.

In 1932, after having spent several months in the tropical surroundings of Singapore (in order to shake off a bout of depression), Hill returned to Cambridge to take up his work on haemoglobin that Hopkins had assigned him. He embarked on a study of this compound's reversible oxygenation; and for doing so he developed precise spectroscopic methods, which enabled him to monitor quantitatively the conversion of haemoglobin to oxyhaemoglobin, and vice versa. "The central problem was how haemoglobin could combine reversibly with molecular oxygen when haematin could not", is how Hill later formulated the goal of his studies.[118] Hill found, among other things, that myoglobin (muscle haemoglobin) had an even higher affinity to oxygen than the usual haemoglobin. Yet, despite these promising findings, the chemistry of related plant pigments, such as chlorophyll, remained in Hill's mind as a field into which, at some point, he still wanted to move. In 1936, Hill finally gave it a try, although he was hardly well-prepared to do so: Hill later believed that he had "crashed in" on the photosynthesis research scene. His biographer Derek Bendall described the situation as follows:

> Armed only with a reading of Spoehr's monograph [on photosynthesis, published in 1926], F. F. Blackman's analysis of limiting factors (he had attended Blackman's undergraduate lectures), and the realization that the path of his own research, where Hopkins had pointed firmly towards blood, led indirectly towards the green leaf. Others in the Biochemical Laboratory had successfully studied oxidation–reduction reactions in cell-free extracts of animal tissue; the same approach applied to leaves was to revolutionize the study of photosynthesis.[119]

The successful use of cell-free extracts by his colleagues in animal biochemistry encouraged Hill to try out the same approach in photosynthesis. If respiration, which had long been considered to be invariably bound to cell structure, could occur in certain suspensions, why not photosynthesis? In his first attempts to prepare an appropriate suspension of leaf extracts, Hill failed to observe any biochemical activity at all, in agreement with the traditional claim that the cell's structure was indispensable. Yet, there was this observation, well-known from Willstätter and Stoll's 1918 monograph, that dry leaf powder was able, for a short time, to produce oxygen, if illuminated. Hill decided that he would follow this up.

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[117] Keilin (1925a). See Keilin (1966) on the history of research into cytochromes.

[118] Hill (1965, p. 124).

[119] Bendall (1994, p. 153).

His persistence paid off. By ingenious, albeit unconventional, means, Hill finally succeeded in preparing a satisfactory suspension of isolated chloroplasts: with a pestle and mortar he ground up leaves of, for example, the common chickweed (*Stellaria media*) and the white dead-nettle (*Lamium album*) in a buffered sucrose solution (pH 7.9) and then filtered them through glass wool. (This was before the ultracentrifuge had become a standard instrument in biological laboratories). Hill wanted to find out under which conditions these chloroplasts were able to produce oxygen. So he added a very sensitive indicator, namely myoglobin, which from his earlier work he knew would be converted into oxymyoglobin in the presence of only minute amounts of oxygen.[120]

Hill found that oxygen was, in fact, produced—yet only if an aqueous leaf extract preparation was added to the suspension. He first interpreted this finding as being due to a lack of certain enzymes, which in the chloroplast extract might no longer be present in their active forms. However, in developing these experiments further, Hill observed that a yeast extract, which certainly contained no plant-specific enzymes, could also promote the release of oxygen, and that the efficiency of the latter was proportional to its content of organic (ferric) iron compounds. Finally, it transpired that oxygen evolution could even be triggered by simply adding to the suspension inorganic iron salts, for example, in the form of ferric potassium oxalate. Catalase-inhibiting agents did not affect the production of oxygen in the system and neither did cyanide. The former was contrary to expectations, given the usual assumption that oxygen was produced in the chloroplasts by the decomposition of peroxides through the action of catalase. Hill was able to demonstrate that the participation of peroxides in this system was highly improbable. However, the most remarkable fact was that carbon dioxide was unable to act as a hydrogen acceptor. While carbon dioxide was the only known substance that could cause oxygen evolution in natural photosynthesis, ferric iron was the only reagent that was able to cause oxygen release in Hill's chloroplast suspensions. This was rather disappointing. It seemed to indicate that the reaction in Hill's extract did not, after all, represent cell-free photosynthesis; and it was completely unclear whether the reaction was related in any way to the process in living plants and algae.

In his 1937 publication, Hill carefully avoided jumping to any rash conclusions. The experiments required enormous skill and circumspection, for example, in order to ensure that the production of oxygen was not due to some property of the myoglobin, and hence were likely to produce artefacts. Another point of concern was the observation that the level of oxygen production was rather low, reaching only about one-tenth of the yield of normal photosynthesis. Nevertheless, Hill became more and more convinced of the validity of his findings, and in 1939 he gave a bold explanation of what his results might imply. First of all, Hill emphasised the fact that the chloroplasts' reaction was not specific to ferric oxalate; the latter was only a

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[120] See Hill (1937, 1939) for the first publications and Bendall (1994, pp. 153–154), for an illuminating retrospective description. Hill himself never used the term "myoglobin" but always spoke of "muscle haemoglobin", which is frequently (and misleadingly) abbreviated to "haemoglobin".

means of demonstrating a general property of the chloroplast. It seemed that ferric oxalate or other hydrogen acceptors were able to oxidise a substance in the chloroplast, which was reduced in the course of the photochemical process and was vital for the release of oxygen. In the words of Hill:

> There must therefore be [in the chloroplast] some primary substance which is reduced [in the light], while at the same time giving oxygen. If this primary substance is A, and the reagent B, such as ferric oxalate, represented in terms of hydrogen transport, we have the following reactions:
> $2\,A + 2\,H_2O \longrightarrow 2\,AH_2 + O_2$
> $2\,AH_2 + B \longrightarrow BH_2 + A$
> [...] It must be concluded that the substance A is not easily removed from the chloroplast because great dilution of the suspending fluid did not diminish the rate of reaction with ferric oxalate.[121]

Hill thus suggested that the chloroplast might contain a mechanism that operated independently of the living cell, "which under illumination simultaneously evolves oxygen and reduces some unknown substance [A] which is not carbon dioxide". Hill assumed that this substance A was a kind of "respiratory catalyst".[122] And it was this substance A that transferred hydrogen to suitable acceptors, such as ferric oxalate, which could therefore be restored to its original state and be used again (while without ferric oxalate, all of this substance would be quickly reduced and the reaction would come to a standstill).

Together with Richard Scarisbrick, who became a long-standing collaborator of his, Hill elaborated and refined these studies over the next year.[123] They were able to show that the low limit of oxygen production, observed in Hill's earlier studies, was due to the reoxidation of ferrous oxalate to ferric oxalate, which consumed a large share of the oxygen that had only just been released. When the reduced compound (ferrous oxalate) was removed from the system, by the additional supply of ferricyanide, the full amount of oxygen released became apparent at high pressure: "The chloroplast then, with ferric oxalate as a hydrogen acceptor, behaves in a similar way to the whole cell as regards the production of oxygen during photosynthesis".[124] The reaction was also demonstrated to be highly sensitive to urethanes, which corresponded to what Warburg had found in his *Chlorella* experiments, and, like during the process of photosynthesis, it was influenced by varying light intensities. In view of these findings, Hill and Scarisbrick felt entitled to conclude that:

> ... the measured activity of the system in the isolated chloroplasts responsible for the production of oxygen in light represents a part of the process of normal photosynthesis. [...] The new conclusion that can be drawn from the work on isolated chloroplasts is that oxygen itself is formed in a photochemical reaction during which there is no reaction involving carbon dioxide.[125]

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[121] Hill (1939, p. 207).

[122] Hill (1939, p. 209).

[123] Cf. Hill and Scarisbrick (1940a, b).

[124] Hill and Scarisbrick (1940a, p. 61).

[125] Hill and Scarisbrick (1940b, p. 254).

This reaction—the production of oxygen by chloroplasts in suspension supplied with artificial hydrogen acceptors—later became known as the "Hill reaction", a term that was coined in 1941 by Charles Stacy French and the Cambridge-based protein chemist Mortimer Louis Anson. (Hill himself never adopted this term but always spoke of the "chloroplast reaction"). French, who at the time was working as a research assistant to James Franck in the Fels Laboratory at the University of Chicago, recalled that Anson had dropped in, on his way back from Arizona to Princeton, "to tell James Franck about Robin Hill's discovery of oxygen evolution by isolated chloroplasts".[126] Anson stayed for a month, and together with French repeated Hill's experiments in many variations, and even improved upon the technique. (They found, for example, that the efficiency of the reaction could be greatly enhanced by working at low temperatures). Franck tolerated these studies, although he believed "that all this had nothing to do with photosynthesis", a widespread attitude at the time.[127] Eventually, French and Anson prepared a paper to be presented during the physiological section of the annual meeting of the Botanical Society of America, which took place from 29 to 31 December 1941 in Dallas, Texas. However, since neither of them was able to attend the conference, their friend and colleague Jack Myers read out the paper to the audience.[128] In this paper, French and Anson explicitly looked at whether the production of oxygen in isolated chloroplasts used "the same enzymes as the oxygen production step in normal photosynthesis".[129] The paper was not exactly a sweeping success. In fact, as Myers later recalled, "it was greeted by a rather stony silence".[130] Some years were to pass before the importance and accuracy of Hill's findings would be realised.

4.6.2 Implications of the Findings

Hill's two main contributions to photosynthesis research of the 1930s were: *first*, he succeeded in separating the photosynthetic production of molecular oxygen from the reduction of carbon dioxide to carbohydrates. By doing so, he provided convincing evidence that these two parts of photosynthesis occurred separately. *Second*, his findings suggested that the photochemical part of photosynthesis comprised the release of oxygen, without carbon dioxide being involved as a hydrogen acceptor. Thus, Hill's experiments strongly reinforced the hypothesis (which van Niel had arrived at

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[126] French (1979, p. 10). The review Franck and Gaffron (1941, p. 219), states, however, that Hill's findings only came to their notice upon publication of Hill and Scarisbrick (1940a).

[127] French (1979, p. 10).

[128] See French and Anson (1941) for the abstract of the paper. In the accounts of this episode in French (1979) as well as in Myers (1974), the name of the society was inaccurately reported.

[129] French and Anson (1941). Incidentally, in these experiments French and Anson were the first scientists to use spinach as a source of chloroplast; it remains a popular source to this day.

[130] Myers (1974, p. 422).

from a totally different starting point) that the photosynthetic oxygen originated from the light-induced hydrogen transfer from water to an appropriate acceptor. Hence, water, and not carbon dioxide, was the source of photosynthetic oxygen. These findings had a marked effect on the field, as can be taken, for example, from Gaffron's autobiographical essay of 1969:

> As late as 1936 Wohl and I were thinking about a hypothetical way to reduce a carbon dioxide compound directly à la Willstätter–Warburg. Only when Hill's chloroplast reaction [. . .] made any other than van Niel's view untenable was I ready to give in.[131]

Besides these conceptual consequences concerning the mechanism of photosynthesis, Hill's achievements opened up completely new avenues in terms of methods and materials. Hill was the first to succeed in preparing in vitro suspensions capable of photosynthetic reactions, which up to then had been considered impossible. Furthermore, Hill's findings stimulated the search for other reagents that might be used as hydrogen acceptors; this eventually led from ferric iron to TPN (i.e. NADP; (see Chapter 7).[132] Finally, Hill singled out not only a biochemical process but also a cellular component—the chloroplast—which subsequently became the subject of a broad range of other biochemical and biophysical studies.

4.7 On the Verge of New Perspectives

4.7.1 Biological Studies Generalised

The period examined in this chapter shows strikingly convergent developments in very different fields of experimental biology, such as physiology, biochemistry and microbiology: they illustrate, *first*, the firm conviction held by researchers at the time that physical and chemical tools, concepts and methods were indispensable for studying life processes (and, hence, had to be included in the curricula); *second*, they reflect the wide-spread searching for broad and comparative perspectives within the life sciences. Biochemists started to take an interest in plants; bacteria began to be used, for the first time, as experimental organisms of value in the study of the metabolism of higher organisms. Biochemical unity at the metabolic level became part of the body of generally accepted knowledge.

Far more people than ever before became interested in photosynthesis research. New research questions emerged, such as clarifying the relationship between photosynthesis in plants and the processes in bacteria; or exploring the physical nature of the energetic transitions in the light reaction stage of photosynthesis. Parallel to this process of ramification of photosynthesis research, one can observe a marked increase in the frequency and popularity of conferences and more informal meetings

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[131] Gaffron (1969, p. 11).

[132] Cf. Myers (1974, p. 422).

on the subject. Many people strongly felt that the problem was much more complex than had previously been envisaged and that a multidimensional approach was required if all the questions on photosynthesis were to be answered. This became a strong incentive for interdisciplinary communication and cooperation—resulting, by the 1940s, in the foundation of the first interdisciplinary research groups to be exclusively dedicated to the study of photosynthesis: notably, the Photosynthesis Project at the University of Illinois at Urbana–Champaign, headed by Robert Emerson and Eugene Rabinowitch; the Fels Laboratory at the University of Chicago, led first by James Franck and later by Hans Gaffron; and, starting in 1946, the photosynthesis division of the Bio-Organic Chemistry Group at the University of California at Berkeley, headed by Melvin Calvin and Andrew A. Benson. I shall come back to these institutions in later chapters.

As was mentioned in the introductory section to this chapter, the 1930s also saw the appearance of the first "professional" researchers in photosynthesis—scientists who developed more than a passing interest in the subject. All the central actors discussed in this chapter belong to this category. It is worthwhile dwelling a little on their career paths, that is, on how they originally came to work in photosynthesis. Two related factors deserve special attention. First, all the major players in this period were educated at institutes or departments that were in the process of eroding or, at the very least, undermining, traditional disciplinary matrices. James Franck's doctoral thesis was supervised by Emil Warburg, whose interdisciplinary interests were discussed in chapter 3, and who inspired Franck to explore the physical basis of photochemistry (while contingent circumstances, such as the lack of an appropriate infrastructure for studies in nuclear physics clearly contributed as well to Franck's shift of research focus). Robert Emerson, William Arnold and Charles Stacy French were all trained in programmes with an emphasis on general physiology, the thrust of which was largely paralleled by the development of general biochemistry, which left its mark on Robin Hill, and general microbiology, which led Cornelis van Niel to study bacterial photosynthesis. These were the disciplines that, during the 1930s, greatly enhanced and fostered the application of physical and chemical methods to biological problems. It was also in this decade that Warren Weaver launched the Rockefeller Foundation's programme to support projects along these very lines (which later he would call "molecular biology").[133]

This intellectual climate was obviously a good preparation for a successful career in photosynthesis studies. However, the spread of the theme was also strengthened by the close interpersonal links between the players—the second factor to be observed among this chapter's protagonists. Gaffron, for example, came to photosynthesis by way of Otto Warburg (he worked as the latter's assistant). Gaffron's interest was

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[133] Of the wealth of literature on this topic, see, in particular, Kohler (1991) and Kay (1993). Having suggested that photosynthesis, which was still considered a marginal subject, greatly profited from the advancement of "new biology"; one could even turn it the other way round and claim that photosynthesis research paved the way for the development of the "new" or molecular biology. See, e.g., the argument brought forward in Zallen (1993b).

fostered even further by the discussions he held with Max Delbrück, Kurt Wohl and other physicists in Berlin, who were all keen to solve the basic problems of the life sciences. Likewise, Emerson and French worked with Otto Warburg for an extended period; and Arnold worked with Emerson. On leaving Warburg's laboratory, Gaffron went first to work with van Niel and finally ended up working with Franck (joined shortly thereafter by French). Eugene Rabinowitch, who will enter the scene fully in the next chapter of this book, worked as an assistant to Franck in the latter's Göttingen days, before becoming Emerson's colleague at Urbana–Champaign. These links also played a role in promoting interdisciplinary discussion.

4.7.2 The Main Lines of Thought

As has become clear, during the 1930s, scientists approached photosynthesis from a number of very different angles and traditions. Figure 4.6 provides an overview of the resulting models and their relationships to each other. With the exception of van Niel and Hill, who came from rather different scientific backgrounds, most protagonists reacted closely to their colleagues' earlier work. At the beginning of this chapter, the relationships between the models leading up to Franck's 1935 suggestion were examined. Gaffron and Wohl harshly criticised this latter proposal, upon which Franck and Herzfeld attempted to find a better solution in 1937. When this proved untenable, Franck and Herzfeld then initiated, in 1941, a new era of models, which were constructed under the assumption that far more than 4 light quanta were available for the completion of the photochemical process—a relief that will be further explored in chapter 5. In the following sections, I shall recapitulate the general development and spell out the rationale behind each of the main lines of research.

4.7.2.1 Fluorescence Studies and a New Standard Model

The first line of thought continued the tradition that was outlined in chapter 2. The Willstätter–Stoll model, seemingly well established from the point of view of chemistry, was taken as the starting point for the analysis of photochemical details. Important new input was provided: first by the finding of Warburg and Negelein (1923) that, in order to produce one molecule of photosynthetic oxygen, no more than four to 5 light quanta were required. Second, there was the suggestion, first made by Kautsky and Hirsch (1931), that the peculiar changes of fluorescence in photosynthesising chlorophyll solutions could be used to analyse the underlying mechanism. The latter was combined with the suggestion that oxygen was, in actual fact, the first hydrogen acceptor in photosynthesis. After their 1918 monograph, Willstätter and Stoll had turned to totally different themes, each working independently: Willstätter had tried out enzyme chemistry, while Stoll had started a career in the laboratories of the Sandoz company in Basle (Switzerland), where he focused on pharmacological questions, such as the chemistry of ergot. In 1932, however, Stoll turned again to

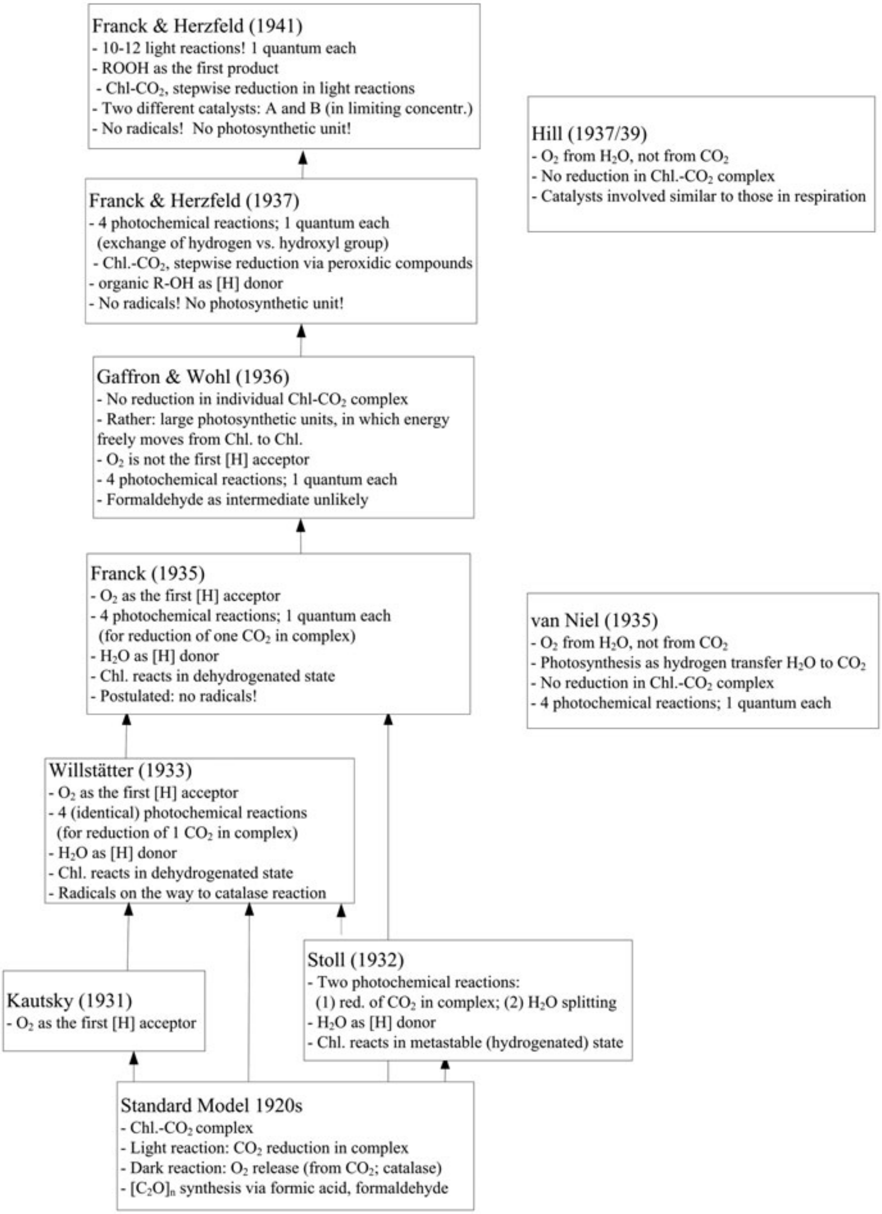

Fig. 4.6 The most important models of the photosynthetic mechanism brought forward in the 1930s and their relationships to each other. Only the main characteristics of the models that are not identical to the standard model of the 1920s have been listed.

chlorophyll—to its structural properties as well as other aspects. The paper discussed earlier in this chapter was mostly an update of the Willstätter–Stoll model of 1918 in light of Stoll's new findings (above all, the discovery of the two loosely bound hydrogen atoms in the structure of chlorophyll) and of the more general development of understanding redox reactions in terms of the transfer of hydrogen. Stoll's suggestion that water be regarded as a hydrogen donor is to be seen in this context.

Perhaps more interesting is the context of the contribution that Willstätter made in 1933. Although Willstätter had mainly written it in response to Stoll's paper, it was also a summary of his 1931 work, carried out with Haber, on the role of chain reactions initiated by chemical radicals in biological processes. During the course of their work, Haber and Willstätter had first explored the possibility of the formation of the HO_2 radical in the context of the catalytic decomposition of hydrogen peroxide in solutions.[134] This paper was cited in the 1933 contribution. Haber promptly reacted by immediately writing a letter to Willstätter, stating how pleased he was that, first, Willstätter had continued trying to solve the problem of photosynthesis, which Haber himself had been unable to sort out, and that, second, Willstätter had employed to this end their common theory of radicals.[135] Taking into account this theory of radicals, Willstätter felt that he could include oxygen as a raw material of the reaction, which was in line with Kautsky's hypothesis. However, neither Stoll nor Willstätter found it necessary to revise their 1918 model completely. Rather, both tried to extend specific parts of the model—different modules – while leaving other segments untouched. This is a fine demonstration of the stepwise extension of a model and explains why, in his short note, Willstätter failed to mention any of the details about the carbon moiety.

Franck's perspective on the problem was clearly shaped by his background in quantum physics: the one empirical finding to which he gave more weight in his work than most of the other photosynthesis researchers was the minimum quantum requirement value proposed by Warburg and Negelein. Franck's early (pre-1941) models were designed, first and foremost, to accommodate this parameter by including four photochemical reactions steps, each of which operated with a quantum requirement of one. This implied that one had to avoid the assumption of radicals and back reactions—not an easy task, to be sure. Yet, Franck was convinced that a photosynthesis model that did not comply with the basic thermodynamical parameters (which were empirically determined) would not survive.

Thus, all three of these scientists pursued research-opportunistic strategies (see Fig. 4.7): having completed some work on the structure of chlorophyll, Stoll took the opportunity to use these findings to contribute to the general problem of the photosynthesis mechanism. The same holds true for Willstätter, although his findings

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[134] See Haber and Willstätter (1931), which argues that biological oxidation should be seen as a dehydration process. The elimination of hydrogen, Haber and Willstätter suggested, usually resulted in the formation of radicals (since only one of the two corresponding electrons would be removed at the same time). Cf. Willstätter (1973, p. 378); Werner and Irmscher (1995, pp. 30–31).

[135] Werner and Irmscher (1995, pp. 122–123); letter from Haber to Willstätter, 24 February 1933.

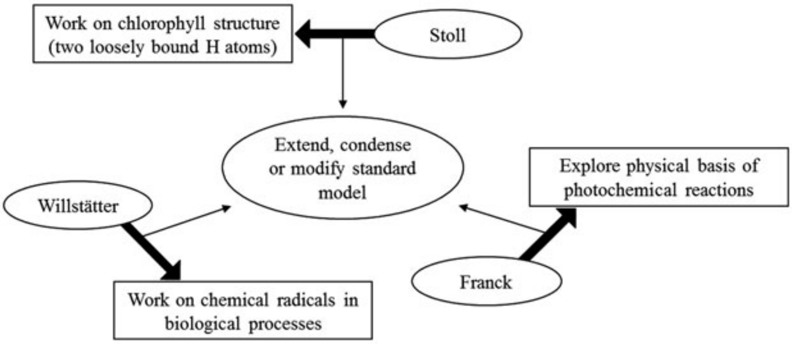

Fig. 4.7 Actors and their goals: diverging individual (superordinate) goals; extending the standard photosynthesis model as a subgoal or incidental goal. The thick arrows in bold typeface indicate the relationship "X pursues the superordinate goal Y"; thin arrows indicate that, in the course of pursuing the superordinate goal, the incidental goal of contributing to finding the photosynthesis model emerged.

were not concerned with chlorophyll, but with the formation of radicals as the central factor of the biological processes that occur in solutions. Franck used his theoretical expertise in fluorescence and energy exchange processes to try and clarify the subject. His suggested mechanism proved particularly influential. All three of them, however, left most of the elements of the standard model untouched: the formation of a chlorophyll–carbonic acid complex as the main reaction site; the reduction of carbon dioxide as part of the photochemical reactions, with chlorophyll as an actual participant; the formation of molecular oxygen as a result of the catalase-driven removal of hydrogen peroxide; and the formation of carbohydrates as a condensation process starting from the formaldehyde units.

4.7.2.2 Flashing Light Experiments and the Photosynthetic Unit

As outlined earlier, Emerson came to photosynthesis via the tradition of general physiology, as well as via Otto Warburg, the supervisor of his doctoral studies. The flashing light experiments of 1932 were intended to clarify two issues: first, a phenomenon that Warburg had noted in passing (that one could increase the rate of photosynthesis by using intermittent light and dark periods); second, the confusing observation, first noted by Willstätter and Stoll, that the rate of photosynthesis was not directly proportional to the chlorophyll content of the photosynthesising agent, as the standard model would have implied. That Emerson hit upon something that would prove to be the downfall of the standard model of photosynthesis as far as the function of chlorophyll was concerned, was, thus, hardly intentional. The resulting paper presented the surprising findings without, though, giving far-reaching interpretations. While Franck produced one conceptual model after the other and mostly played the role of theoretician in the history of photosynthesis research, Emerson was the empiricist. One could interpret this as a matter of personal style and preference; different types of people tend to pursue different lines of research, whichever are more to their liking and talent. However, it is also a matter of education and knowledge. Franck simply lacked the necessary experimental skills to handle algae and manometers; and Emerson was not a quantum physicist. Yet, the same individuals may be able to play different roles in different contexts: Franck started off as an experimentalist in physics, not as a theoretician; it was only in photosynthesis research that he kept to theory.

The case of the photosynthetic unit nicely illustrates how different background knowledge and earlier experience can lead to different interpretations. In order to account for the low ratio of one molecule of oxygen developed per several thousand molecules of chlorophyll, Emerson raised the possibility that the enzyme necessary to process the photochemical products might be present in very low concentrations. This was the factor, he thought, that was responsible for the low ratio of the end product. This was an entirely reasonable assumption, given the maxim that one should try and keep to the established knowledge of the time for as long as possible. Franck, on the other hand, shaped his theory with Herzfeld as the inverse of autooxidation processes. These he had studied intensively earlier in his career, for example, in 1931 together with Haber (shortly before the latter turned to investigating radicals with Willstätter).

Gaffron and Wohl designed their bold explanatory hypothesis against the background of the Delbrück colloquia. Their discussions were driven by the belief that in biology totally new and unexpected kinds of processes (or even laws) could be found if the insights of quantum physics were applied with sufficient competency. From this perspective, the suggestion that the photochemical reactions in photosynthesis might require the cooperative action of thousands of molecules was just what Gaffron, Wohl and their Berlin colleagues had been searching for. However, the hypothesis was not enthusiastically received by other parties. Gaffron and Wohl had summoned up convincing arguments against the standard model (it consumed too much energy; the assumption of very long-lived intermediates was unfounded; much longer induction periods would be required). But they were unable to present a mechanistic description of how the cooperation of chlorophyll molecules might work. Also Gaffron and Wohl took the Warburg–Negelein value of the quantum yield for granted; and neither did they question the fact that the oxygen had to originate from carbon dioxide via peroxidic compounds. The only part of the standard model that Gaffron and Wohl attacked was the assumption that there existed a chlorophyll–carbon dioxide complex in which the latter was reduced in a one-to-one relationship. The further pathway of the reduced carbon moiety remained largely untouched.

4.7.2.3 Microbial Photosynthesis and the Generalised Equation

Far more fundamental was the challenge that arose from microbiology, a field of study that traditionally had been far closer to medicine than to biology. It was only thanks to the Microbiology Department of the Delft Technical College in the Netherlands, where van Niel had trained, that the discipline got off the ground and that the importance of microbial investigation became recognised by other subfields of biology. From the available evidence, van Niel seemed to have pursued two goals: first, he wanted to find out more about the fascinating diversity of microorganisms; and second, and almost as importantly, he wished to use his knowledge of general microbiology to elucidate the fundamental problems of metabolism, independent of the research organism of choice. The fact that he chose the *Thiorhodaceae* as experimental organisms should not be overrated—chance clearly played a role here. When van Niel started working as Kluyver's assistant, the latter was preparing a lecture course, which, among other themes, also touched upon iron and sulphur bacteria. Van Niel's first task was, therefore, to prepare adequate cultures of these organisms for demonstration purposes. In order to do so, van Niel had to familiarise himself thoroughly with these difficult and heterogenous groups; and in the course of this work, he discovered that there were striking phenomena in the metabolism of sulphur bacteria about which a number of conflicting explanations had been claimed, none of which was entirely convincing. In addition to this spur, van Niel recalled that he "had become enamored with the aesthetically attractive purple sulfur bacteria".[136]

Notwithstanding all these contingencies, through his immersion in general microbiology van Niel was, without question, extraordinarily well prepared to conceptualise the metabolism of the purple sulphur bacteria and to compare them with green sulphur bacteria and plants. The basic assumption of metabolic and biochemical unity was not a consequence of his studies but a presupposition. The striking similarity between the summary equations of the processes in bacteria and plants or algae (they mostly differ in their use of appropriate hydrogen donors) was enough to convince van Niel of the existence of a general photosynthetic process. However, few in the scientific community were ready to accept his conclusion: after all, processes which come down to the same summary equation—i.e. the same behavioural description—can proceed by entirely different mechanisms. Van Niel's observation was merely that purple sulphur bacteria (as well as some other bacteria) were able to reduce carbon dioxide in the light, while at the same time oxidising some substances (mainly H_2S) in the medium being used. In order to call this "photosynthesis", one had to accept that the release of oxygen was not a defining feature of photosynthesis, which was quite a step, even for microbiologists. It seemed particularly audacious to assume this basic unity of process in view of the broad range of reactions observed in bacteria, which adapt so rapidly to changing environments, and the unchanging photosynthesis in plants and algae.[137]

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[136] See van Niel (1967, p. 9).

[137] This discrepancy was later taken by Franck and Gaffron (1941) as an argument for the assumption that "the anaerobic type of photosynthesis is the same in all cells but that it is supplemented in green plants by the capacity of liberating gaseous oxygen. [...] Photosynthesis in plants, therefore, is the exception to the general rule" (p. 252). By contrast, van Niel (1941) assumed that in all types of photosynthesis water is reduced, while in bacteria the liberated oxygen immediately underwent

The most important implication was that one had to take very seriously the assumption that the oxygen produced during photosynthesis originated from water and not from carbon dioxide. Accepting this consequence would not only have made the standard model of photosynthesis untenable, but also implied that the neat balance between the volumes of carbon dioxide consumed and of oxygen produced suddenly was merely coincidental, which to many researchers was hard to believe.[138]

4.7.2.4 Oxygen Evolution in Chloroplasts

It was Hill's work that eventually helped dispel the reservations scientists had about van Niel's hypothesis: light-driven oxygen evolution by chloroplasts was possible without there being any need for carbon dioxide reduction. It was emphasised earlier how carefully Hill made sure that his observations were not mere artefacts but reflected the photosynthetic processes under natural conditions. Of course, evidence for this assumption was not fully conclusive. Like van Niel, Hill argued for the hypothesis that a process observed under circumstances X (isolated chloroplasts; bacterial metabolism) was the same as a similar process under circumstances Y (illuminated chloroplasts in plants), so that both could be described by the same model. Reservations were strong: even for most of the 1940s it was still being debated whether all the photosynthetic oxygen really did come from water. The absence of a convincing mechanism to achieve the decomposition of water (which required a very strong reducing agent) made people rather doubtful of the validity of this hypothesis.[139]

4.7.3 A New Conception of Photosynthesis

In addition to all these steps towards a new model of the photosynthetic mechanism there was also an important change on a more fundamental level, that slowly crystallised during the decade looked at in this chapter and deserves a moment of attention. It began to dawn on the researchers involved that the process was much more complex than previously imagined. More factors than anyone would have imagined were found to be of influence on the process—in particular, the physiological state of the experimental organism and its developmental history. The growth conditions

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secondary reactions and the dehydrogenation of the specific hydrogen donor took place in later stages of the process.

[138] Today it is known that this "neat balance" is no longer tenable and the quantities as well as proportions can vary substantially.

[139] It was Sam Ruben and Martin Kamen's experiments in 1941 with "heavy" water, incorporating the oxygen isotope ^{18}O, that provided the strongest evidence for the hypothesis that photosynthetic oxygen came exclusively from water. See Ruben et al. (1941) for the publication, which will also be discussed in Chapter 6.

of the algae turned out to be highly relevant (such as exposure to light, pH value, atmospheric pressure, carbon dioxide concentration, and so on). Different genera and species of algae, also closely related strains, were shown to react quite differently to changes in conditions; and even when the same experimental organism, cultivated under the same standard conditions, was investigated, the data tended to vary.

The enormous flexibility of photosynthesising organisms was addressed most explicitly by Hans Gaffron in a paper of 1940.[140] Therein, he emphatically pleaded for a move away from the traditional, quasi-mechanical concept of photosynthesis, which (unlike respiration!) still was assumed to have a fixed stoichiometry. The latter was based on the long-standing assumption that the ratio of carbon dioxide consumed to oxygen released was unity, which had given rise to the hypothesis that carbon dioxide was the source of oxygen. Gaffron pointed out that this ratio became unity only under stationary conditions, while particularly at the points of transition of, for example, light to darkness, very different ratios were obtained. Further complications arose if one considered the interference of photosynthetic processes with many other reactions in the cell. The oxygen released during photosynthesis, for example, might be immediately consumed again by oxidising intermediate respiration products or by other reduced compounds of the metabolism.[141] The result would be that none or only a part of the oxygen was liberated while varying amounts of carbon dioxide were formed. Yet, how researchers were to deal with these complexities, how they ought to reorganise their work in the laboratory as well as their inferences and interpretations, Gaffron was unable to suggest.

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[140] Gaffron (1940).

[141] This shift in the conception of photosynthesis neatly coincides with a general change in biochemistry. Up to the 1930s, a conception of metabolism as a set of linear processes prevailed. However, when ATP and various coenzymes were discovered in the 1930s, scientists slowly realised the close entanglement of the cell's metabolism, which came to be conceived of as a highly integrated system; cf. Bechtel (1986a).