Of Light and Darkness

Modelling Photosynthesis 1840-1960

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Chapter

INTRODUCTION

Scope of the Study

This book examines the process of a scientific discovery that took more than one hundred years to reach completion. It is a study of light and darkness – metaphorically speaking, in terms of dark failures and bright successes, and in a very literal sense, referring to the chemical effects of light and its absence. Today photosynthesis is known as the process by which solar energy is converted into energy that can be used in biochemical reactions. It is fundamental to life on earth, and the way organisms accomplish this task has intrigued scientists for centuries. The first tentative (and rather simplistic) ideas were developed by organic chemists around 1840, but it was only from the 1920s onwards that noticeable progress was made in understanding the mechanism. In particular, great strides were made after 1945, and by 1960 an elaborate photosynthesis model at a molecular level had been established. This model included a set of light reactions, with two different photochemical systems, which was linked to a light-independent sequence of dark reactions via a cyclic pathway. Almost immediately the model became widely accepted, and in the decades that followed work on extending and refining it would dominate the field.[1]

This modelling of the complex process of photosynthesis involved the contributions of many international research teams that consisted of actors from very different disciplinary backgrounds; a broad range of experimental techniques were employed and very different modelling approaches were proposed. The study of how the process of photosynthesis was modelled can thus be taken as a case in point for the beginning of large interdisciplinary research programmes, which have since become the norm in science organisation. I argue that, notwithstanding the inevitable competition that exists between research teams, the process can best be described as the (mostly informal) cooperative efforts of a research collective,

[1] A fair body of literature can be consulted on the subject, much of it contributed by the actual scientists involved. For treatments of the history of photosynthesis research in otherwise science-oriented books, see, e.g., the pertinent sections in Rabinowitch (1945) and Loomis (1960). Photosynthesis is also touched upon in general works on the history of biochemistry, such as, e.g., Fruton (1999) (pp. 325–329), although these sections are comparatively short and sketchy, since the focus of these books is usually on the biochemistry of human metabolism. For specific timelines and surveys of the history of photosynthesis research, see, e.g., Myers (1974), Höxtermann (1992), Huzisige & Ke (1993), Gest & Blankenship (2004), Govindjee & Krogmann (2004) and Nickelsen (2008*b*). The work of Govindjee (one name only) deserves special mention: he has been promoting studies in the history of photosynthesis research for many decades now and he played a pivotal role in introducing a "Historical Corner" in the journal *Photosynthesis Research*, in which tributes, obituaries, mini reviews and personal perspectives are published. Together with a number of different co-authors, Govindjee edited, in the early years of the twenty-first century, three issues *Photosynthesis Research* that were entirely dedicated to the history of the subject; see Vols. 73 (2002), 76 (2003) and 80 (2004). The papers in these issues have more recently been collated into a seminal volume entitled "Discoveries in Photosynthesis"; see Govindjee, Beatty, Gest & Allen (2005).

which, by dividing the labour, step by step pursued a common goal: to elucidate the biochemical and biophysical mechanisms of photosynthesis by establishing a causally explanatory model. From this perspective, the scientific modelling of a process is regarded as the collective search for an accurate and adequate causal graph. In this sense, this book also tries to re-emphasise the dictum that the history of science without the philosophy of science is blind, while the philosophy of science without the history of science is empty.

How Science Works

Imagine a young and aspiring scientist, in the second half of the 1930s, say, who has just finished his first degree in a branch of biological sciences. As he had chosen one of the more progressive universities of the time, he had, in addition to plant and animal morphology, also studied a fair amount of physiology, chemistry and even physics – and had become fascinated by the functioning of the metabolism of living beings. Since he found himself unable to develop a taste for the practice of animal physiology (which seemed to include, among other things, a fair amount of animal dissection), he decided to specialise in plant sciences. The scientist remembered, perhaps, that, in his course of elementary botany, it was touched upon (if only in passing) that plants were able to "photosynthesise", that is to reduce carbon dioxide to sugar by utilising the energy of sunlight, and thereby produce oxygen. Other details of the process had been mentioned in the course, although, as far as the student could remember, its main point was that most aspects of photosynthesis were still totally obscure. This sounded like a promising starting point for an ambitious doctoral thesis!

Assume that the young scientist then approached the professor who had given the lecture on photosynthesis, and discovered that not only was he one of the few experts in photosynthesis at that time, he was also the head of a research group devoted to the subject. The senior scientist was delighted to find somebody who was interested in joining the project, agreed that he would be able to master a challenging project and immediately assigned him one. The task was, say, to find out whether the rate of photosynthesis in the red alga *Porphyridium cruentum* changed at different temperatures and with different pigment content; and the professor advised him to go and learn manometry and the basics of algae treatment from the laboratory technician over the following few weeks.

What a sobering experience! The aspiring scientist had probably envisaged that he would: ponder the theme at length with the head of the group; select the most fascinating question that needed clarifying (preferably discovering the whole mechanism at his first attempt); work out the necessary methods; pick the instruments from the shelf; and then go on to make exciting new discoveries. On the contrary, before he had even had time to think about the project, everything (in terms of method and content) had already been fixed; and, even worse, his project seemed to concern questions of only marginal interest. How could the temperature preferences of an inconspicuous red alga possibly be of relevance to the Big Question – the mystery of how photosynthesis worked? Why was the head of the group unwilling to think of methods other than the ones he had been using during the past few decades?

I shall now leave the (purely fictitious) young scientist at this frustrating point in his career. It is highly likely that, in the course of his work, he eventually learned to understand more fully his role in the project of modelling photosynthesis. He would have learned that it was impossible for any one person to attain the big goals, and that these goals were divided into sub-goals that had to be reached on the way; that one was able to form hypotheses on the basis of very preliminary experimental data; and that the widespread phenomenon of remaining with one experimental method and one single conceptual approach was not necessarily because of any lack of intellectual flexibility or an indication of senility. As so much knowledge and so many competencies were required to make one valuable contribution to this field, it was imperative that researchers selected questions that could be solved on the basis of the know-how available to them. I will now examine these issues in more detail as well as introduce the systematic background of this study.

Epistemic Systems

This study's approach was formed by the concept of *epistemic systems* that Gerd Graßhoff developed in the 1990s and that has been constantly elaborated to this day. The fundamental assumption is that scientific research should be regarded as a complex network of problem-solving activities, and that this network is governed by superordinate and subordinate goals, heuristics and methodological rules.[2] Epistemic systems are thought to comprise all the components that are relevant to a research process. These include notably: (i) *epistemic goals* (wishes to perform an act that directs the researcher to the solution of his or her problem); (ii) *propositional attitudes* (beliefs, assumptions or considerations – notwithstanding their actual truth); (iii) *heuristics* (rules of action according to which either propositional attitudes are generated, for example, a belief in a causal hypothesis, or physical actions are performed, for example, an experiment is carried out); and (iv) *epistemic actions* (cognitive or physical acts that constitute the actual steps of a research process). Epistemic systems also include the materials requiremd to perform the actions, that is, the necessary instrumentation and manpower. Finally, epistemic systems are thought to be dynamic entities, that is, they are in a state of flux as actions are performed, background knowledge grows and as techniques and instruments are elaborated. The interaction of different elements of an epistemic system is governed by the so-called "principle of action". In its basic shape, this principle was proposed by Paul M. Churchland in 1970,[3] while Graßhoff refined his variant by: (i) differentiating between elementary and complex actions and establishing how they relate to each other; and (ii) framing the conditions for action in terms of causally relevant factors, instead of taking the whole principle to be a material implication.

The reconstruction of the discovery of the urea cycle by Hans Krebs and Kurt Henseleit is the historical case study that decisively shaped this approach. In this episode of experimental research, which involved six months of labour by

[2] See Graßhoff (1994) in which the approach was first developed; for its application to historical case studies, see also, e.g., Graßhoff (1998*b*), Graßhoff (1999), Graßhoff, Casties & Nickelsen (2000) and Graßhoff & May (2003).

[3] See Churchland (1970), p. 221.

two collaborating scientists, not only was the hierarchy of goals, which drove the biochemical research in different stages of the project, identified; the process of hypothesis formation was also explained by way of a regularity theory of causation and causal reasoning, which succeeded in marvellously accounting for the actors' actual reasoning at the time, as far as it is documented in the fully preserved laboratory notebooks kept by Krebs and Henseleit.[4] The first extension of this approach to accommodate episodes to which a larger number of scientists contributed was Graßhoff's reconstruction of how the astronomical object SS433 was modelled after having first been observed in 1978.[5] The current study on the development of photosynthesis research draws strongly on the experience gained from these earlier projects.

Goals, Sub-Goals and Actions

Epistemic systems help to explain why scientists do certain things. There is little doubt that scientists "do" things all the time: they build instruments, they take readings, they design experiments, they sit around and ponder their data and, finally, they write up a paper. The approach of epistemic systems goes one step further and assumes that, most of the time, scientists do all these things with definite goals and purposes in mind – notwithstanding the fact that these goals and purposes might be in a state of flux. Scientists not only *do* things; they perform *actions*, the reasons for which, according to traditional action theory, are to be found in their goals and beliefs.

The actors in this study all shared the common goal of attempting to explain the process of photosynthesis. However, it was clearly impossible for any one single person working at any one moment to achieve this goal. Among many other things, explaining photosynthesis would have meant that one had to find out how chlorophyll absorbs light and channels it into chemical reactions, how carbon is reduced to carbohydrates, how photosynthetic oxygen is released, and so on. Explaining photosynthesis required that all these problems, and many more, be solved. Solving each of these problems was, so to speak, a "subordinate goal" (or sub-goal for short), the achievement of which was crucial if the actual, superordinate goal was to be reached. But even these sub-goals, such as finding out how chlorophyll absorbs light energy, were still too complex to be accomplished at one attempt, so that they had to be divided again into further sub-goals. By this means, a large and nested hierarchy of goals unfolded; and the most any one scientist could hope to achieve was to succeed in contributing small pieces to the

[4] For a first reconstruction of the episode that describes Krebs as a successful "tinkerer", see the relevant chapter in the biography of Krebs by Holmes (1991). An alternative reconstruction is given in Graßhoff et al. (2000), Graßhoff & May (2003) and Nickelsen & Graßhoff (2008). The laboratory notebooks, which fully document Krebs's and Henseleit's experiments on urea synthesis, were published as Graßhoff & Nickelsen (2001) and Graßhoff & Nickelsen (2002). The latter also comprises a synopsis of all the experiments undertaken by the two protagonists, combined with an analysis of the goals of the experiments and their role in the whole episode. Finally, a computer program, based on the aforementioned theory of causal reasoning, was developed and implemented (first in early versions of Prolog, later in a version of Java) that succeeded in simulating the course of the discovery; see, e.g., Graßhoff (1998*a*) in addition to the publications already cited.

[5] See Graßhoff (1998*b*).

whole, that is, attain some of the sub-goals necessary to explain the complete process of photosynthesis. There is nothing unusual in this process; most other research themes in the sciences are pursued in the same way – which is one of the reasons why science should be considered a totally cooperative enterprise. Scientists are obliged to cooperate with one another, even if most individuals would prefer to make their own discoveries. They have to take note of the findings of their fellow scientists, because in many instances knowing the results of other scientists' experiments is crucial to one's own project. In this study, scientists who are connected to each other in this manner – that is, by sharing common research goals – are called members of a research "collective".[6]

The unfolding of a nested hierarchy of goals also has an effect on the way that the work of individual scientists is organised. Imagine a biochemist who has to set up an algae culture in a growth medium with a definite pH value. To reach this goal she must, among other things, make an appropriate buffer solution. Therefore, she has to get a beaker, borrow an instrument, pick up the ingredients from the shelves, mix everything together and then measure the pH. If she finds that she cannot attain any one of these necessary sub-goals – if, for example, there is no bicarbonate available – she would need to overcome the pertinent obstacles before continuing with her higher-ranking goal: that is, before the biochemist could carry on with setting up the algae culture, she would have to go and borrow some bicarbonate from a neighbouring laboratory, *or*, if this proved impossible, order some from the laboratory shop.

The fact that there exist a number of alternative actions that seem appropriate to reaching a goal, as in this simple example, is very typical. There are, of course, external restrictions on the set of alternative actions from which scientists can choose; it may be, for example, that all the biochemist's colleagues were on holiday and had locked up their laboratories for a fortnight. Comparable situations arise if the instrument for the optimal method is unavailable; if there is not enough time to use the most precise measuring technique; or if the scientist simply does not have the necessary knowledge and competencies to process the statistical data effectively. Yet even then, there usually remain a range of viable alternatives. In a collective, such as the photosynthesis researchers as a whole, or only those who were concerned with chlorophyll studies, the different alternatives available to attaining a goal might well be attempted by different individuals or even by different scientific teams; and the actions that were needed to be performed to reach a sub-goal might be assigned to colleagues working in the same laboratory. Remember the aspiring young scientist's project to find out whether the rate of photosynthesis was dependent on temperature or pigment content? The head of the group had assigned to him a certain red alga as a research organism, while his colleague in the laboratory perhaps pursued the same question using the green alga *Chlorella*. Both projects might, in the end, contribute to the laboratory's larger superordinate goal

[6] The discussion about "collectives" immediately brings to mind the classic study, written in 1935, by Ludwik Fleck, who was the first to start thinking about *Denkkollektive* and *Denkstile*; see Fleck (1994) for a fairly recent edition of the text. However, the way "collectives" are understood in this study differs from Fleck's notion, since my only assumption is that members of a collective share the same research goals, although they might or might not share additional conventional assumptions (which was an essential part of Fleck's concept).

to arrive at a comprehensive kinetic model of photosynthesis. And while in this laboratory the method of choice was manometry, there were other laboratories that were also trying to find the kinetic model of photosynthesis, but by other means, such as microcalorimetry. Thus, in many cases the acts undertaken by the people working in a laboratory can be taken to represent the nested hierarchy of actions and goals, which usually comprises a range of alternative options.

Choosing between alternatives requires a criterion to determine the order of preferences. The biochemist would probably first go and see whether the neighbouring laboratories had any bicarbonate before he went on to order it from the shop, since the former option would be much easier and faster. But these are not the only aspects to consider; sometimes the more complicated action might be preferable – in this case, for example, one also has to consider the purity of the substance. How to pick the most attractive option from a range of alternatives – given one's own situation, resources and competencies – is no easy decision. In this study, I shall argue that in many cases the scientists were guided by a handful of general "heuristic principles" – rules of thumb that they acquired during their education. Before scientists embark on their own projects, they have usually undergone a thorough and comprehensive training period, during which they not only acquire the body of generally accepted knowledge within a certain domain and the necessary practical "know how" but also a set of useful heuristics that advise them how to achieve their epistemic goals. These heuristics are not infallible and they might not necessarily lead scientists to the best solution, but they do provide a set of well-proven recommendations, which most scientists are inclined to follow.

If one observes photosynthesis research as a whole, a surprisingly large number of those realistically alternative actions were, in fact, attempted in many of the cases looked at in this study. This could only be achieved by dividing the labour between as many heads and hands as possible, since photosynthesis researchers tended to work in a rather limited domain: they focused on a certain sub-goal (such as finding the kinetics of photosynthesis), used a certain technique (such as manometry) and persevered with it for a large part of their working lives. But if the knowledge and competencies of all these experts were put together, then there was a much better chance of covering a wide range of subjects and approaches. Which sub-goal and which method an individual scientist chose for himself can usually be explained by examining a scientist's education and career. Many of the actors in this study were introduced by their supervisors and mentors (whose importance has long been acknowledged by historians of science) not only to a certain discipline but also to a certain order of priorities and a certain method. All the aspiring young scholars who spent some time in the laboratory of Otto Warburg, for instance, used manometric techniques for the rest of their working lives – not because they were unable to think of anything else but because, since they had learned to master one of the best techniques available at the time, there was little incentive to try out alternative methods. Which theme a scientist chose to address with these methods, however, also depended on the range of themes they had worked on in other laboratories. In addition, a considerable number of players fell into photosynthesis research from a different field of study, thereby importing their expertise in terms of concepts and methods, in order to contribute

to a specific sub-goal that seemed attainable with their package of competencies. And while some of them returned to their original field of study, others found the sub-goal more complex than expected and thus stayed in photosynthesis research.

Few of the actors examined in this study radically changed their methods and their principal notion of how photosynthesis had to be addressed conceptually in the course of their professional careers. James Franck, for example, who had come from the realm of quantum physics, remained reluctant for the rest of his life to accept that the photochemical process in photosynthesis diverged from everything he had learned studying inorganic processes. This apparently inflexible and ignorant behavioural pattern is frequently seen as a sign of intellectual stagnation. Yet, while this may well be true in some cases, one has to be aware that such a judgment is founded on unreasonably high expectations. Sometimes the silent assumption, if only expressed between the lines, is that one individual scientist should be able to master all the available methods, interpret all the data from all angles at the same time and, hence, make the big discovery on his or her own. This is clearly asking too much. The history of photosynthesis research, like the history of most other scientific subjects, is not the story of a couple of individual geniuses: no single person discovered the accurate photosynthesis model in a solitary moment of illumination. It is the story of individuals, who contributed to the common goal: everybody was an expert in something but nobody was an expert in everything. There were, of course, some scientists who were better than others, either in terms of providing comprehensive interpretations or of performing outstanding experimentation. Yet, in their work, too, they depended on the many pieces of knowledge that had been accumulated thus far by others.

Experiments and Causally Explanatory Models

None of the actors of the story I am relating had any doubt about what it meant to "explain" photosynthesis: they were searching for a sequence of causally relevant factors that acted together to bring about the effects in question: the production of carbohydrates and oxygen. In this study the whole set of factors that brings about effects is called a *causally explanatory model*. The importance of scientific models has long been acknowledged in the philosophy of science; indeed, attempting even an approximately adequate review of the literature would be a project in itself.[7] Over the past fifteen years this debate has widened to include the discussion about "mechanisms", particularly in the field of biology. The claim is that scientists often refer to mechanisms, that is, to a specific collection of entities and activities, in their attempts to explain certain phenomena.[8] To the extent that these mechanisms are intended to causally explain sequences of events, which most of them are, they

[7] The debate was initiated in the 1960s by studies such as Black (1962) and Hesse (1963). Since then, the literature has proliferated enormously. A useful overview is provided, e.g., by Frigg & Hartmann (2006). Morrison (2006) also summarises parts of the debate and discusses the value of theories in comparison with models. Bailer-Jones (2009) gives a survey of how models were treated in the history of philosophy of science, as well as an analysis of what a scientific model constitutes (as compared to, for example, a theory).

[8] The literature on "mechanisms" and several aspects of their discovery and function in science is rich: see, e.g., Machamer, Darden & Craver (2000), Bechtel & Abrahamsen (2005), Glennan (2005), Craver (2006), Bechtel (2006), Bogen (2008) and Darden (2008). See, however, Weber

resemble the models under discussion in this book; however, the disadvantage of the mechanism approach is that, unlike causally explanatory models, mechanisms do not have access to a rich causal reasoning framework, which greatly helps one to analyse experiments and to transfer experimental results into a causally structured model from the point of view of the philosophy of science.

The models in this study are explanatory in so far as they spell out the relevant factors which produce the effects in question and define their relationships to each other. Sometimes this can result in the description of rather complicated structures, for example, in cases where the factors act in sequences that comprise many different steps, which, in turn, are intertwined with each other, so that the model resembles a network rather than a linear sequence. The relationship between relevant factors and their effects is taken to be of a causal nature, the explication of which does the explanatory job. I take these models to be causal graphs – abstract entities that can be aptly represented as directed networks of nodes and edges. In most of the chapters of this book, a standard mode of representing causal graphs is applied to the representation of the models in question.[9] The main advantages of this standard notation are that: first, it greatly clarifies the models' content, making it more easily accessible; and, second, it makes it much easier to make comparisons between the different models. (Note, however, that the notation becomes limited as soon as the models become too complicated and require different diagrammatic representations. In Chapter VI, therefore, the representation chosen by the actors themselves was frequently retained. The choice of means of representation does not, however, affect the form of the abstract content.)

This study's models were constructed to explain biochemical – and biophysical – processes, at different levels of explanation. Scientists usually call these explanatory models "pathways" or "mechanisms". Biochemical pathways describe the stepwise development of products out of a series of reactants; they may take the form of a long chain of reactions or the form of a cycle.[10] They are often very complex, although for many purposes simplified versions work well, as they can be extended as occasion and knowledge demand. As is the case for every causal graph, biochemical pathways are necessarily incomplete; the absence of a factor in the modelled pathway does not denote the factor's irrelevance. In short, biochemical pathways are causal graphs; and it is interesting that these pathways are frequently depicted in graph form even by the actors themselves. Fine exemplars from the case studies examined in this book are the Calvin–Benson–Bassham Cycle, which describes the so-called dark (thermochemical) reactions of photosynthesis, and the Z-scheme, which describes the light (photochemical) series of reactions of the process (although the latter should, perhaps, be called a "biophysical pathway").

(2008) for an argument as to why mechanisms are not necessarily required to provide (causal) explanations of natural phenomena.

[9] For the underlying theory of causation and causal reasoning, see, e.g., May (1999), Graßhoff & May (2001) and Baumgartner & Graßhoff (2004). The latter also provides an accessible introduction to the representation of causal processes in the form of graphs. The extension of this theory to the analysis of experiments is provided, e.g., in Graßhoff et al. (2000).

[10] On the reconstruction of the discovery of the urea cycle in the framework of a regularity theory of causation, see, e.g., Graßhoff & May (2003) and Nickelsen & Graßhoff (2008).

Such models do not emerge fully-fledged overnight. As mentioned in the previous section, it can take a great many actors months, years, sometimes decades of labour to construct a model. It is this process that is the focus of this study. Long-term studies of the processes of model construction are still rare, even though they are indispensable for addressing some unresolved historical and philosophical questions.[11] As I explained earlier, it is assumed in this study that the construction of these models can be regarded as a sequence of actions intended to solve a scientific problem. The best way to construct such a model is to find out the causal connections between the involved factors: the construction of an explanatory model is, to a very large extent, the search for causal relationships and causally relevant factors. This can only be done by conducting difference tests, which are usually carried out in the form of experiments.[12] A relationship of causal relevance between two factors can be established from these tests if the following is true: (1) the two situations fulfil the homogeneity condition, that is, they are appropriately similar in so far as (a) all necessary co-factors are present, so that the causal relevance of the testing factor would be discerned; (b) the effect is not brought about by alternative causes; (2) an effect E is produced if a factor A is realised, while E is not produced if A is absent. The causal link between A and E will then become part of the body of established knowledge, so that one can justifiably add a new edge to the graph under construction (and perhaps also an additional node). Causal inferences are deductive inferences, that is, they are truth-preserving; and if the premises are known to be true, the conclusion has to be true, now and in the future. (Note that the truth of the causal inference necessarily hinges on the truth of the homogeneity condition. Establishing the truth of the latter is far from easy but not impossible.) For example, it had already been established in the eighteenth century that carbon dioxide was causally relevant to the photosynthetic production of oxygen in green plants, and later generations of scientists could not possibly have ignored this piece of knowledge. This is one of the main reasons why modelling has to be a conservative process: scientists simply cannot afford to neglect the thus far accumulated knowledge of the causally relevant factors involved.

The Modelling Process

This study also looks at how the scientists constructed complex graphs from the inferences they made from the results of difference tests: even if a factor is demonstrated to be causally relevant to a certain effect (for example, carbon dioxide is causally relevant to the production of molecular oxygen in green plants), the kind of influence it exerts might still be uncertain. Usually, some causal knowledge is taken as the prototype, which can be a rather simplistic one. For example, it may be that some substances are known (or strongly suspected) to participate in a pathway, although it is unclear how they are related to each other. This was the situation when the team centred around Melvin Calvin and Andrew A. Benson at the University of California, Berkeley (United States) started modelling

[11] To this day, there are very few studies available with a similar focus; see notably Graßhoff (1998*b*) and Bailer-Jones (2000).

[12] See Graßhoff et al. (2000) for an introduction to the analysis of experiments from the point of view of a regularity theory of causation.

the dark reactions of photosynthesis on the basis of tracer studies and paper chromatography in 1946. The scientists did the following: (1) they identified those substances that are present in the greatest amounts (since they will most probably be the major players); (2) they found out as much as possible about the reactions that the substances in question usually undergo; (3) they specifically looked for possible sequences of steps that might lead from one of the identified substances to another; and (4) they double-checked all these options to see whether evidence for the occurrence of one (or more) of the potential pathways could be produced in further experiments. Additionally, the group worked under the usual assumption that photosynthesis should function, *mutatis mutandis*, along the same lines as similar reactions that were already better known.

This type of situation is the rule rather than the exception in science. Put in more abstract terms, the group started with a simplistic prototype model that included some of the main factors that would bring about an effect and then tried, step by step, to expand this prototypic causal graph. Each expansion had to be tested for the validity of the hypothesised causal relationships; and subsequent findings might have led to the preliminary model hypothesis being modified.[13] In doing so, at least three general heuristic methods played a role: the first was the application of causal knowledge that had been gathered from elsewhere – perhaps from completely different fields of investigation; second, as many alternative models as possible were pursued at any one time (even if some of them looked initially unpromising); and, third, a modularisation technique was employed for the graph under investigation by identifying the partial processes, which were either ignored or contained a plug-in description of whole sequences of relevant factors, using background knowledge. These principles will be explained in more detail in Chapter I, and will appear in later chapters too.

Finally, note that, although the actors in this story are assumed to have pursued definite goals at all times and it is argued that they did employ general methodological principles and follow heuristic rules that were widely accepted, this does not imply that investigational histories, of individuals or of the whole collective, are linear and predictable. Neither does this last fact render them inexplicable. Inevitably, there remain some indeterminacies, where the surviving sources do not allow one to reconstruct adequately the reasons behind certain actions; but it is the aim of this book to limit these indeterminacies as far as possible. In order to do so, the epistemic goals and working hypotheses of the actors are given centre stage. Although techniques do play a central role and do restrict the range of available (and attractive) alternative actions, in the cases under discussion here, they do not determine the course of research to the extent that is assumed, for example, in the "experimental systems" approach that was developed by the historian of science Hans-Jörg Rheinberger.[14] The story that is told here was mostly problem-driven (which does not deny the existence of coincidences and chance). Instruments were important and the choice of appropriate model

[13] These basic modelling actions are described in more detail in, e.g., Graßhoff (1998*b*).

[14] See, e.g., Rheinberger (1997) for the publication that initiated the so-called "experimental system" approach to analysing research episodes in terms of the fate of epistemic things and systems.

organisms was of great significance. However, they were not the motors of research; they were the vehicles.

I will now explain how the main body of this text is organised.

Overview of the Book

In Pursuit of a Pathway (1843–1918)

Chapter I concerns itself with the attempts made in nineteenth-century Europe to reconstruct the process of photosynthesis. The body of knowledge was still rather scant then, and the only methods that were available for metabolic studies of this type were inadequate. Nevertheless, the subject still attracted attention from many different quarters. I will demonstrate how different research goals resulted in the formation of different research collectives, which were determined by the actors' competencies and interests. Focusing on the chemical collective, I present the two main proposals that were advanced to explain photosynthesis: Justus Liebig's organic acid model and the formaldehyde model proposed by Adolf von Baeyer. A large portion of the remaining chapter is then devoted to the study of how these models were received and modified by the collective. Several hybrid versions were developed, all of which mixed a different set of the building blocks that was assumed to be part of the eventual solution. Many of these versions were pursued for a surprisingly long time, even though some of them did not appear at all promising; as even the most favoured versions were not built on entirely safe ground, researchers seemed to consider it worth their while to examine all the possible options. Finally, it is argued that every one of these models had a definite focus that was not a mere consequence of the common goal; rather this focus can be explained by referring to the individual background of those scientists who proposed the models. In this early phase of modelling photosynthesis, most, if not all, of the people contributing to the research did so on a "part-time" basis: they had other research goals, and their contributions to photosynthesis research was a by-product of the other work that they were pursuing. This finely illustrates the principle of research opportunism, which is an important heuristic strategy: one makes contributions to a developing field as long as it does not distract from one's actual research goals for too long.

Otto Warburg and the Turn of Manometry (1912–25)

Chapter II takes a completely different perspective. Instead of observing a collection of collectives, I focus my attention on a specific individual: Otto Warburg, arguably the most brilliant (and most successful) biochemist of the twentieth century. Although Warburg is primarily known for his work on cell respiration, he devoted a large part of his working life to questions related to photosynthesis, his first contribution to the subject being published in 1919 and the last in 1969.

Warburg's contributions to this field are manifold: first, and of most lasting importance, he introduced a new set of methods, including manometry, bolometry and the study of unicellular algae as research organisms, into the field of photosynthesis, and this had a revolutionary effect on photosynthesis research in subsequent decades; second, Warburg and his long-standing collaborator Erwin Negelein were the first to study the energetic requirements of the photosynthetic

process; and third, and least known (and of little lasting effect), Warburg proposed a new model to explain how the separate processes of photosynthesis operate, based on the data gathered in manometric experiments. The aim of this chapter is to analyse the nested hierarchy of research goals that brought Warburg to this series of contributions. It becomes clear that the priorities of each actor within a research collective are strongly influenced by personal contacts, education as well as by chance. Warburg, as a case in point, came to study photosynthesis, first, because of his wish to extend his work on cell respiration to other energy-producing reactions; and second, because of the inspiration he had gained from working with his father, Emil Warburg, in photochemistry. For his own model hypothesis Warburg transferred his earlier ideas on respiration to the standard model of photosynthesis of the time (as represented in the work of Richard Willstätter and Arthur Stoll). These were the principal sources of Warburg's "building blocks", which he extensively used to construct his photosynthesis model. Warburg's impact was enormous, and he will continue to play a role in the subsequent chapters of this book, not only because of the new techniques that he introduced but also because of his role in the question of the maximum quantum yield in photosynthesis – an issue that began about fifteen years after the first measurements that Warburg and Negelein had undertaken and that developed into a long and acrimonious controversy.

Warburg's work was also instrumental in re-organising the distinct photosynthesis collectives. Warburg held doctoral degrees in both chemistry and medicine, so that he was extremely well qualified to carry out the metabolic studies for which he became so famous. Warburg pursued the same general goal as the nineteenth-century chemists – he strove to reconstruct the biochemical pathway of photosynthesis, from the raw materials to the end products – but he held decisively different views as to how to reach this goal. Warburg was used to working with living cells, so the advantages of working with unicellular algae instead of either artificial, inorganic systems (as the chemists did) or the much more complex higher plants (as plant physiologists did) were self-evident to him. Furthermore, Warburg was sufficiently interested in physiological approaches in general for him to endorse in his work the shift from looking for chemical intermediates (for which no methods were available) to the study of the kinetics of the process (for which manometric methods were ideal). And, finally, Warburg greatly enlarged the methodical and intellectual repertoire of the photosynthesis collective by bringing the field into contact with experimental and theoretical physics, from which most of his contemporaries in both chemistry and plant physiology were rather detached.

Struggling With The Standard Model (1930–41)

The crossing of disciplinary borders that Warburg exemplifies – the combining of goals, beliefs and methods that formerly were held by very different collectives – became the model for photosynthesis researchers of the next generation, who are dealt with in Chapter III. By the end of the First World War at the very latest, it was strongly believed that the field of biology was in urgent need of reform. Up to then, the biology curriculum had been dominated by morphology and taxonomy, while physiology or a general scientific education, including introductions to chemistry, physics and mathematics, were mostly absent. Many of the more

physiologically oriented biologists felt that this was not an adequate preparation for young scientists hoping to solve the pressing problems of the day – such as, for example, animal and plant metabolism. In most cases the attempts at reform failed to initiate major changes (the curricula at most universities only began to change after 1945); yet, in some places the debate did yield innovative results. The introduction of new subjects of study and fields of inquiry, such as general physiology, general microbiology and (general) biochemistry greatly promoted the study of photosynthesis. Remarkably, all the major players in the 1930s came from contexts that were at least amenable to the setting up of these new and more general science-oriented directions in biology or to the cross-fertilisation of disciplines. Photosynthesis researchers with this sort of background included, among others, William Arnold, Robert Emerson, Charles Stacy French, Hans Gaffron, Robin Hill and Cornelis B. van Niel.

Although the decade of the 1930s was a period of exciting new discoveries in the field of photosynthesis, it proved extremely difficult to integrate them into a coherent explanatory model. The "standard model", agreed upon in the 1920s, was still accepted by most scientists as a satisfactory working hypothesis (if surely not the last word). In light of new developments, the model was modified and extended, for example, by Willstätter and Stoll, but also by James Franck, who in this decade turned his interest to the physical foundation of photosynthesis. However, a number of experimental findings were presented that proved difficult to reconcile with the traditional approach. Among the most important novelties in this period were: first, the observation that the ratio of molecular oxygen produced to the ratio of chlorophyll molecules present was about 1:2500, which led to the suggestion that there might be a "photosynthetic unit" – the assumption that the chlorophyll pigments of a plant might cooperate in a way previously unheard-of; second, the finding that not only plants but also certain bacteria were capable of reducing carbon dioxide to organic substances in a light-driven reaction, and the subsequent proposal of a "general equation for photosynthesis"; and, third, the successful isolation of chloroplasts, which could be triggered to produce oxygen without the presence of carbon dioxide. The latter two findings endorsed the hypothesis that, contrary to commonly held assumptions, the oxygen that is produced from photosynthesis originates not from carbon dioxide but from water. However, no one was able to formulate a mechanism that would lead to the oxidation of water in the necessary way; and even though, by the early 1950s, it was widely accepted that the source of the oxygen produced in photosynthesis is water, not everybody was convinced of this (as no appropriate mechanism had yet been proposed). So, because of these new discoveries and the slowly accumulating data on the energy requirements of the process, the standard model of the time ran into trouble; and no convincing alternative was in the offing.

The atmosphere of change in the 1930s clearly had an effect on the general development of the collective as a whole. Far more people than ever before became interested in photosynthesis; new sub-goals emerged, such as clarifying the relationship between photosynthesis in plants and the processes in bacteria, or exploring the physical nature of the energy transfers in the light reactions. Parallel to the broadening of this field of research in terms of new themes, problems and

approaches, one can observe a large increase in the frequency and popularity of conferences and more informal meetings on photosynthesis. Many people strongly believed that the problem was much more complex than had previously been envisaged, and that a multi-dimensional approach was required if a solution were to be found. This proved a great 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 (all in the US). The most notable were: the Photosynthesis Group at the University of Illinois at Urbana–Champaign, headed by Robert Emerson and Eugene Rabinowitch; the Photosynthesis Laboratory at the University of Chicago, headed by James Franck with his collaborator Hans Gaffron; and, starting in 1945, the photosynthesis division of the Bio-Organic Chemistry Group at the University of California at Berkeley, led by Melvin Calvin and Andrew A. Benson.

The Maximum Quantum Yield Controversy (1937–55)

Chapter IV looks at the controversy surrounding the maximum quantum yield of photosynthesis. The debate started when, in the late 1930s, American research groups revisited the quantum requirement of the process and found a minimum requirement of eight to twelve quanta for the photosynthetic production of oxygen, which was clearly at odds with the standard value of four to five, which had been proposed by Warburg and Negelein back in 1923. The discussion of this discrepancy was postponed because of the outbreak of the Second World War but resumed after 1945. Warburg was then invited to the University of Illinois in the US, to the laboratory of Robert Emerson, who was one of his former doctoral students, with the idea that they sort out the problem together. The maximum quantum yield was, after all, an important modelling parameter of photosynthesis: in fact, it was one of the only parameters that constrained the range of potential models, so that settling this question was considered to be of great importance. However, no agreement was reached while Warburg was at Urbana; rather, he left in anger, never spoke a word to Emerson again, and the whole debate disintegrated into an extremely unpleasant dispute. With hindsight, it is hard to believe that so much time and energy was spent on this question. In particular, the controversy had the effect of inhibiting Warburg and Emerson from making any further contributions to other aspects of photosynthesis. Ironically, then, although Warburg spent so much time on photosynthesis, he made few contributions to elucidating its mechanism from the point of view of his actual field of expertise, that is, from the realm of biochemistry.

The course of events was unproductive, to say the least. It started with Emerson raising some precise critiques of Warburg's experimental protocol. However, instead of trying to answer the criticisms directed at his approach by demonstrating the causal relevance of those factors that he held to be responsible for the extremely high yield in his experiments, Warburg randomly changed the set of experimental conditions under which he had been able to measure a maximum quantum yield of four (or even three, as time went by, which was the thermodynamical maximum). Warburg never explained why the new set of conditions was causally relevant to inducing the algae to such high efficiencies; as a result, Emerson was obliged to go through the new methods again so as to find the sources of error. A decisive

turn of events came about in 1955, when Emerson presented a comprehensive article, based on a vast amount of new data, in which he explained why, under certain conditions, Warburg had been able to measure very high quantum yields, which were produced as a result of experimental artefacts (resulting mainly from Warburg's use of a phosphate buffer solution as a medium). Warburg, however, seemed not the slightest bit disturbed and continued to discredit his opponents, accusing them of arguing on theoretical terms only, while he himself "asked nature" in his experiments. The majority of the informed collective, however, was highly impressed by the explanations that Emerson provided. It was clear to everybody that this was not a trivial question: the experiments to measure quantum yields in biological contexts were problematic and demanding, so for a long time there was much uncertainty about who was right (contrary to popular belief, the long period of disagreement was not only due to the scientific community's blind faith in Warburg's authority). Value set against value was insufficient and unsatisfactory; however, the situation changed when Emerson was able to provide sophisticated explanations as to *why* Warburg had measured these (wrong) results.

Yet, Warburg refused to give in, and for this reason Emerson also continued to pursue the issue. The only (lucky) discovery Emerson made while trying to detect the Warburg group's experimental errors was that of the so-called Enhancement Effect, which he observed in the second half of the 1950s. Earlier, Emerson had found that, at long wavelengths and at low light intensities, photosynthetic efficiency dropped dramatically, although the absorption of chlorophyll remained high. He now observed that if one combined this long wavelength light with light of shorter wavelengths the rate of photosynthesis increased far more than if the light intensities had been merely added together. At the time, nobody knew how to explain this finding, but it paved the way for the later introduction of the two-photoreaction, two-pigment model of photosynthesis. Emerson would not live to see the fruits of his discovery harvested: he died, in 1959, in an aircraft crash. Learning of Emerson's death, Warburg expressed relief that at least now the controversy was over. Although this was, in large part, true, contrary to what Warburg might have believed, it had not been settled in his favour. Based on other pieces of evidence, around 1960 the photochemical events in photosynthesis were successfully modelled as a series of two light reactions that occur in two different pigment systems; as a result quantum yield was downgraded to a question of marginal importance.

The whole controversy is a fine example of, first, how a collective reacts if there are found to be incompatible experimental results. Since it is impossible to directly *falsify* one of the results, the only way out of this impasse is to demonstrate the causally relevant factors that produce the differing outcomes. This is the path that Emerson took but which Warburg ignored. Several attempts were made to solve the controversy amicably; and it is clear that the actors were rather perplexed by the fact that Warburg refused to cooperate in the usual manner by demonstrating the relevance of his approach, either in papers or by experimenting together, in which the different methods could then be directly compared. Instead, Warburg repeatedly insisted that a committee should be formed to solve the argument *ex cathedra*, once and for all. In the end, the collective ignored Warburg's further contributions. By not pointing out the relevant points in his experimental set-up,

Warburg had failed to validate his results and, as a consequence, his colleagues lost their patience with him. Undoubtedly, Warburg had also made himself extremely unpopular through his arrogant behaviour, his polemic style of writing and his failure to acknowledge the findings of other research groups. However, the main point was that his contributions increasingly failed to stand up to the standards of the field.

Elucidating the Dark Reactions (1937–54)

In Chapter V, the discussion moves on to the path of carbon in photosynthesis, that is, to the reconstruction of how carbon dioxide is reduced in the dark reactions of photosynthesis (which, of course, quickly come to a stop in complete darkness as they are crucially dependent on the light reactions of the process). Work on this question was done in parallel to the maximum quantum yield controversy, but by this time the photosynthesis collective had already divided itself rather neatly into two: those who were mainly dealing with the photochemical aspects of photosynthesis and those who were trying to elucidate the chemical fate of carbon dioxide during the process. The successful pursuit of this latter research goal was made possible by the discovery, in 1940, of the radioactive isotope carbon-14, by Samuel Ruben and Martin Kamen, two young chemists at the University of California, Berkeley, who were working under Ernest O. Lawrence at the Berkeley Radiation Laboratory. However, Ruben died in a laboratory accident in 1943, while Kamen was dismissed from his post because of his alleged involvement in un-American activities. (It took him ten years to establish his innocence in court.) So, after the Second World War it was the research team centred around Melvin Calvin, Andrew A. Benson and James A. Bassham that worked on carbon-14 in photosynthesis. The main body of this chapter focuses on the activities of the Calvin–Benson–Bassham team – a large research collective, which not only pursued one single research goal but did so in the same laboratory in an exceedingly open, cooperative and successful manner. Crucial to the sweeping success of this group were, among other things: the intelligence and talent of the scientists; its unusual size and organisation (it was one of the first truly interdisciplinary research groups working with enormous efficiency and verve towards one single research goal); the availability of unlimited financial resources provided by the Atomic Energy Commission (AEC); and the team's use of paper chromatography. Originally developed in 1944, this technique had, in its early years of existence, not been highly valued by biochemists; however, in combination with carbon-14 and radioautography, it proved extremely useful for establishing the intermediate products of the pathway of carbon dioxide reduction.

The project took eight years of intense labour. The Berkeley group's activities are well documented: in extended interviews with project members that were undertaken in the 1990s as part of the University of California's Oral History Project; in the many autobiographical accounts of former members; and in a series of twenty-three papers, all of which were entitled "The Path of Carbon in Photosynthesis". Each of these papers comprised new ideas on how the path was to be modelled, so that this episode provides a rich case study in how step-wise modelling, based on empirical findings and the interpretations thereof, can eventually arrive at a fitting solution. (Melvin Calvin was awarded the Nobel Prize

in Chemistry in 1961 for advancing the final cycle; the indispensable contributions made by other members of the group, in particular Benson and Kamen, regrettably went unrewarded.) The enormous progress made at Berkeley could not be matched by any of the other competing teams – including, for example, a group working under Hans Gaffron in Chicago. The Berkeley team's procedures exemplify all the principles of collective model building that were outlined earlier: its members carried out the principle of model pluralism; they ignored the surfeit of empirical data at any given time; and they undertook step-wise, conservative modelling, based on the findings of difference tests and on the careful transfer of causal knowledge from other fields. The large size of the team made it possible for its members to pursue the full range of potential leads, many of which turned out to be dead ends or red herrings; but in contrast to the search for the value of the maximum quantum yield, all the participants were brought back, in due time, to more productive lines of research, so that they could again contribute to the communal, superordinate goal of the group.

Elucidating the Light Reactions (1950–61)

The final chapter of this story looks at how the light reactions in photosynthesis were elucidated, culminating in the two photoreaction, two pigment system model, which to this day dominates the field. The development of molecular biology also played a significant role: the introduction of new spectroscopic methods was of enormous importance in this context, since for the very first time it became possible to nail down the changing redox states of individual molecules, in response to, for example, the onset of illumination. With hindsight, pieces of information, which would eventually contribute to the two-photosystem model (although, of course, at the time this was not obvious to the participants, who mostly felt that they were groping helplessly in the dark), were gradually accumulated during the decade.

By 1954, it had been established that chloroplasts were able to form the reducing equivalents and the adenosine triphosphate (ATP) that were necessary for the reactions of the carbon dioxide reduction cycle to take place. The challenge was then to find a model that would explain how this was possible, given the fact that the theory of the oxidation of water, which somehow had to be brought about by the use of sunlight, was thermodynamically extremely unpopular. Around that time, two cytochromes were identified that seemed to be specific to the green parts of plants; and when it was observed that one of them changed its redox state upon illumination, some of the central actors were inspired to emulate the successful interpretation of the respiratory chain and find an appropriate electron transport chain in photosynthesis that involved the action of these cytochromes. The understanding of the light reactions of photosynthesis was further advanced by studies in how chlorophyll and the long-suspected "photosynthetic unit" functioned. The new spectroscopic methods, such as the technique developed by the Dutch biophysicist Louis N. M. Duysens, demonstrated that there was a curious transfer of energy between the photosynthetic pigments, which always seemed to terminate at chlorophyll *a*. This apparently had some special function in the process and, in addition, there seemed to be more than one species of chlorophyll *a*. On top of all these findings came the discovery, in 1957, of the (aforementioned) Emerson Enhancement Effect, which strongly pointed to the assumption that two different

photoreactions were initiated through pigments absorbed at different wavelengths. It took only another three years before rather similar two photoreaction models of the primary processes of photosynthesis were reached independently by a handful of actors, who up to that point had been following very different research paths. The eventual model, which subsequently became known as the "Z–scheme" (after a standard form of representation), had, around 1960, surely been "in the air" for some time: all the necessary information was publicly available, and all that was needed was someone to put it all together.

The Z–scheme proved extremely successful and triggered a wealth of new research activities that was initiated by research teams from a broad range of disciplines. New collectives were formed, new research goals emerged, while others were revised in view of the new developments. In terms of explaining photosynthesis at the molecular level, the Z–scheme was only the beginning; for this study, however, it marks an appropriate end point. Although the isolation of historical episodes is always, to a certain extent, arbitrary, this convergence of thought – documented in the Z–scheme, in combination with the Calvin–Benson–Bassham Cycle (which by then had become generally accepted) – can be regarded as being the main achievement of the common research goal that was followed in this study: to establish an accurate and empirically adequate causal graph for the process of photosynthesis. Disagreement about the details, no doubt, still prevails and will never cease which is one of the fascinating things about science. Work on modelling the process continues to this day.

Chapter I

IN PURSUIT OF A PATHWAY (1843–1918)

This chapter examines the attempts made in Europe from the middle of the nineteenth century to the early years of the twentieth century to identify and explain the numerous processes involved in photosynthesis. As the body of knowledge at the time was rather scant and the methods available for metabolic studies were inadequate, it proved extremely difficult for scientists to construct models that explained the chemical steps involved. Over the whole period under study (1843–1918), the metabolism of animals and plants was basically a black box, the internal mechanism of which was totally obscure. Even as late as 1925 the plant physiologist Walter Stiles admitted, in his monograph on the subject, that "the nature of the intermediate substance or substances formed in photosynthesis is a subject on which [...] our real knowledge is practically negligible".[1]

Nevertheless, photosynthesis attracted the attention of a number of scientists working in a variety of fields. The period analysed in this chapter stretches from 1843, the year in which the German organic chemist Justus Liebig published his photosynthesis model – the first attempt to model photosynthesis in terms of a (rudimentary) chemical pathway – until 1918, the date of publication of the voluminous (and seminal) monograph compiled by Richard Willstätter and Arthur Stoll. The latter model, albeit with a few minor alterations, remained the favourite (and standard) account of photosynthesis for years to come.[2] However, after 1918 the main focus of photosynthesis research shifted, not least because new techniques, which enabled a completely new approach to be adopted, became available.

1 Accepted Body of Knowledge

It might seem strange to define the body of knowledge that was "generally accepted" during the whole period under discussion; yet, although this body of data grew enormously over the seventy-five years covered in this chapter, the commonly held core assumptions about photosynthesis went largely unchanged. The well-known equation for oxygenic photosynthesis formulates a large share of these facts:

$$6CO_2 + 6H_2O \xrightarrow[\text{green plant cells, light}]{} C_6H_{12}O_6 + 6O_2 \tag{I.1}$$

This equation, which was defined in the nineteenth century, contains the basic aspects of photosynthesis that were considered acceptable: that carbon dioxide and water are the raw materials of the process (mostly taken together as carbonic acid), and that, in the green cells of plants, they are then converted into carbohydrates

[1] Stiles (1925), p. 193.

[2] Manning (1938) still maintained, many years later, that "it is usually assumed that formaldehyde is the first product of photosynthesis and that subsequent polymerization is responsible for the formation of glucose or other carbohydrates". See Chapter III for a discussion of the development of the standard model during the 1930s.

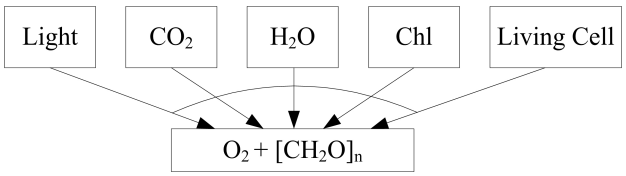

Figure I.1: The elementary one-step model of photosynthesis: the only consensus on the mechanism of the overall process, which remained unchanged from the mid-nineteenth century until the beginning of the twentieth century.

and oxygen under the influence of light. If one includes some additional knowledge, which is not captured in the equation, the undisputed facts of the process were, at that time, as follows:

- Carbon dioxide and water are absorbed from the atmosphere and the soil and are the raw materials of the process.
- Oxygen and carbohydrates are the products of photosynthesis, and are formed by means of a reductive synthesis from molecules of carbon dioxide.
- Chlorophyll pigments play a crucial role in the process by absorbing light energy and making it chemically available.
- As soon as the cell was damaged, photosynthesis stopped; thus, the "living cell", or some then unknown specific aspect of it, was also a necessary factor.

This generally accepted body of knowledge on the mechanism of photosynthesis can be summarised using a simple, one-step model, which is visualised in graph form in figure I.1 (p. 26). The scientists working at the time knew, of course, that more than one step was required to reach the final stage of photosynthesis; however, since they neither agreed on the order of the processes involved in photosynthesis, nor on the question as to which of these processes were light driven and which were not, none of their suggestions can be included in a reconstruction of the body of knowledge.

In this model, molecular oxygen and carbohydrates, the general chemical formula of which is $[CH_2O]_n$, are taken to be the effects of a causal process in which chlorophyll, the cell's structure, carbon dioxide, light and water act as causally relevant factors. They are, at the same time, the "root factors" of the model, since they were taken as the starting points for the process being investigated. Nobody actually believed that this model represented more than a tiny fraction of the whole picture; but everyone was aware that, in whatever way the more complex model would be drawn up, these basic causal connections had to be accounted for – which not only makes this prototypic model a useful starting point for the subject of this chapter but also made it the starting point for all the investigations of the photosynthetic process itself in the period under study.

2 Research Goals and Research Collectives

For a standard body of knowledge to be defined, there must exist a group of people who are the bearers of this knowledge, in this case those scientists who were searching for an accurate and adequate causal graph to explain photosynthesis. Clearly, the graph had to be accurate, because the scientists involved wanted to find the true causal connections between the relevant factors; it also had to be adequate, so that the factors could be determined in acceptable detail and the pertinent empirical data explained.

However, it was clear to everyone that the underlying causally explanatory model of the overall process of photosynthesis would be an extremely complicated one. Even if the process were formulated simply, for instance, as the conversion of carbon dioxide to carbohydrates and oxygen in the illuminated, green parts of plants, at least three very different aspects of the process had to be considered: first, the role of the green parts of plants, that is, the structural prerequisites of the process and their function in relation to leaf tissues and chloroplasts; second, the role of light and how it was able to drive the reaction; and, third, the biochemical pathway leading from the raw materials to the end products. Each of these areas needed to be investigated from a particular perspective by applying a certain set of competencies and techniques, which no individual scientist working in the nineteenth century could hope to master singlehandedly.

Therefore, it is not too surprising to find that there existed a number of fairly distinct groups that were investigating certain specific aspects of photosynthesis. (See figure I.2, p. 28 for a schematic representation.) For example, the structural prerequisites of photosynthesis were studied by plant anatomists and morphologists, who tried to find out, for instance, how chloroplasts were organised and where they were distributed in a plant. Hugo von Mohl, for example, found that the green colour of plants, which up to then was thought to be distributed diffusely in the plasma, was in fact concentrated in distinct bodies, which could take on a number of varied forms and also contained starch (these bodies later became known as "chloroplasts").[3] Work carried out towards the end of the nineteenth century, notably by the botanist Gottlieb Haberlandt, helped bring awareness of the close relationship between the morphology of the photosynthetic mesophyll cells and the way they function.[4] The effects of light, on the other hand, were mainly the domain of physicists, who were interested in light absorption and the chemical efficiency of light, and who were predominantly inspired by the new technique of photography, with which the early photochemical studies were closely connected.[5] Finally, the biochemical mechanism was the sphere of chemists, who are the focus of the rest of this chapter, while the plant physiologists, if they were at all interested in photosynthesis, concentrated on the influences of several macro-parameters on the process, such as light intensity, temperature and carbon dioxide concentration. This list is far from complete, and the assignment of research themes to disciplines should not be taken as absolute. Julius Sachs, for example, who has frequently

[3] See von Mohl (1837).

[4] See, e.g., Haberlandt (1881) and Haberlandt (1884).

[5] See, e.g., Boberlin (1993) for useful background information on nineteenth-century quantitative photochemistry.

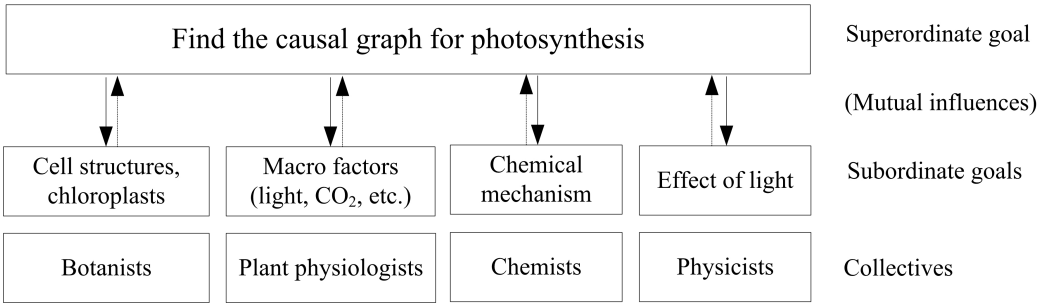

Figure I.2: The nineteenth-century photosynthesis collectives.

been called the "founder" of modern plant physiology and who was most definitely not a chemist, contributed enormously to elucidating the photosynthetic pathway with his discovery, in the early 1860s, that the amylum in chloroplasts was the first visible product of photosynthesis.[6] And researchers from a number of disciplines tried to find out more about the pigments that were involved in the process – an area that I have not included in the figure for reasons of simplification. However, it should still suffice to provide a general idea of the field of study and its many subdivisions.

I shall argue that this quasi labour-dividing organisational practice was not a coincidence. The superordinate goal – to explain the process of photosynthesis – could obviously not be reached by any one person working alone. So subordinate goals (or sub-goals) were defined, the attainment of which would contribute to reaching the main, overarching goal. If one wanted to explain photosynthesis, one had first to explain the chemical effects of light, the working of plant pigments, the range of intermediate substances, and so on. These sub-goals were defined by groups of scientists that were competent in the respective area. In this study, the groups that shared common research goals are called "collectives". In addition to sharing a goal – or rather a host of goals, since goals always come in packages – the members of a collective were usually connected to each another by a variety of means of communication (personal meetings, correspondence, publications, and so forth). Thus, a collective is a communication community, the members of which hope to attain at least one common research goal.

Since there are different hierarchies of goals, clearly there are also different hierarchies of collectives. That is to say, we can regard all those scientists who were trying to explain photosynthesis as members of one large collective, while those whose aim was only to elucidate the chemical effects of light, for example, would be part of another collective, which was not necessarily a mere subset of the former: not all the physicists who were trying to explain the chemical effects of light did so because they wanted to elucidate the different processes involved in photosynthesis. Many of them only indirectly contributed to achieving this superordinate goal – and many of them never came into any form of contact with the scientists involved in other photosynthesis-related activities. In the nineteenth

[6] Sachs (1862) and Sachs (1864).

century, the collectives were rather detached from each other and scarcely noticed each others' results. The separate strands of investigation that were mentioned earlier, especially those connected rather tightly to certain disciplines, proved considerably stable, even beyond the period under investigation in this chapter. Methods certainly changed – for instance, over time electron microscopy became the morphologists' instrument of choice – but the general goal of finding out the structure of chloroplasts remained the same. However, while the strands remained stable, the gaps between them became increasingly filled with overlapping research interests. In the twentieth century more and more scientists actively began to seek interdisciplinary cooperation, a result of which was the founding of hybrid disciplines, such as "biochemistry" and "biophysics". At the same time researchers strove to make their interpretations coherent and consistent with those of other subgroups; however, in the period examined in this chapter this development was only in its infancy.

It should be added that sub-goals and the superordinate goal are, of course, closely interrelated. It is obvious that the superordinate goal had an effect on how the sub-goals were defined. However, the influence might also work in the opposite direction: the work carried out in order to reach a sub-goal, and the insights gathered along the way, could well exert considerable influence on how the superordinate goal was conceived, and this, in turn, might have consequences for the other sub-goals. It is also possible that the superordinate goal was only formulated after work had been done for a while on themes that, with hindsight, could be framed as sub-goals. An example is the research work carried out on the processes of light absorption in nineteenth-century physics: only after some time did it transpire that the findings on this subject were also relevant to the study of photosynthesis. Suffice it to say that we are dealing with a hierarchy of goals and that groups of people had the same goals; and even if these people were working on very different sub-goals, they might still help to accomplish a higher, superordinate goal and so could thus be considered members of the same collective.

3 The Chemists: Goals, Means, Problems

I shall now concentrate on the work of those chemists who investigated photosynthesis in the nineteenth century. In this section, I shall describe in more detail the goals of the collective of chemists, the means of achieving these goals and the problems the chemists encountered.

The primary goal of the chemists involved in photosynthesis research was clearly defined: to reconstruct the biochemical path from the raw materials to the end products of the process. From the point of view of chemistry, this entailed answering two questions (or attaining two sub-goals):

(i) How is carbon dioxide, a highly stable compound, reduced?

(ii) How are the one-carbon units, which are presumably the result of the carbon dioxide reduction, joined together to form large molecules such as carbohydrates?

Whoever contributed to reaching these goals can be considered to be part of the collective under consideration here. The means, of course, included using the body

of knowledge of photosynthesis as described earlier in this chapter (in section 1, p. 25.). In addition to that, the chemists involved had: recourse to a certain body of specific knowledge concerning chemical mechanisms and reaction types; a common understanding of experimental methods; as well as recourse to a set of norms that adequately explained what the problem would look like – it would certainly be more detailed than the one-step model introduced earlier, but nobody would have dismissed a suggestion because it did not outline the molecular details. Yet, the chemists also shared some problems. One major difficulty at the time was the dearth of methods for directly identifying the intermediates of the path. All the information on the course of the biochemical mechanism had to be gathered from indirect sources. The second problem was that chemists were not used to dealing with biological phenomena. In the second half of the nineteenth century, the realm of living organisms was only beginning to enter the field of chemistry, with chemists discovering the potential of these questions for their own work. It is important to bear this in mind as it partly explains why chemists were so quick to postulate all kinds of reaction types, whether they were physiologically sensible or not.

In sections 4 and 5 of this chapter, the alternative approaches developed by the nineteenth-century chemists involved in photosynthesis research will be described in terms of content, evidence and their relationship to each other. Although some of these alternative approaches are well known – notably Adolf von Baeyer's formaldehyde model – no in-depth comparative analysis of the photosynthesis models of this period has yet been undertaken, so that some detail is required.[7] The original publications have served as the source for the models, which I have reconstructed in the form of causal graphs. Note, however, that for practical reasons the selection has been restricted to those models that: (1) were extensively debated when they were proposed; (2) were still considered to be worth mentioning at the end of the period under study; and (3) attempted to cover the full range of chemical processes that were known to be involved in photosynthesis – that is, carbon reduction, oxygen release and carbohydrate synthesis. I have, therefore, excluded those approaches that, for example, dealt only with the action of chlorophyll as well as those in which little thought was given to the general chemical mechanism of the process.

4 The Main Alternatives

4.1 Justus Liebig and the Organic Acid Model

It was the eminent German chemist Justus von Liebig, renowned for many discoveries, who first put forward a possible pathway for the process of photosynthesis. In the context of this chapter it is especially interesting that he is considered the actual founder of what is today called "organic chemistry", which he interpreted as encompassing agricultural chemistry and chemical physiology as well. At the time Liebig was predominantly concerned with developing the foundations for an

[7] See, e.g., Florkin (1977), pp. 147–151, for a discussion of the formaldehyde hypothesis. This theory is also treated in Rabinowitch (1945), pp. 255–260, in which Baeyer's approach is compared with Liebig's point of view.

"agricultural chemistry", which was the context in which he started thinking about photosynthesis.[8]

In 1843, Liebig surmised that organic acids might be the intermediate substances on the path of carbon from carbon dioxide (CO_2) to carbohydrates.[9] A reconstruction of the resulting photosynthesis model in the form of a graph is given in figure I.3 (p. 33). (Note that all the causal graphs in this chapter are only made up of the principal components of the models and are not to be read as representations of stoichiometrically exact equations. No attempt was being made to include all intermediates and factors.) Starting from the observation that fruits gradually sweeten as they ripen, Liebig proposed that various organic acids, such as oxalic, malic, tartaric and citric acids, were the intermediates in the stepwise reduction of CO_2 to carbohydrates:

> If one considers that unripe fruit, for example, grapes, cannot be enjoyed due to their high acid content; that in sunlight these fruits behave in the same way as leaves, namely, that they are capable of absorbing carbonic acid and releasing oxygen (DE SAUSSURE); that at the same time as the acids decrease, the sugars increase: in view of these points, one cannot reject the idea that the carbon of the organic acids in unripe fruit becomes part of the sugars in ripe fruit; that, therefore, the acid is transformed into sugar, effected by the release of oxygen and the components' absorption of water.[10]

Liebig conjectured that the tartaric acid in grapes, the citric acid in lemons, the malic acid in apples, and so on, might be the intermediates on the path of carbon from carbonic acid to sugar: because of appropriate temperatures, light or other factors, the acid that was still present at a certain point in time had not yet been transformed. And since all these acids were usually found in leaves in the form of their ions combined with the ions of alkalis, such as potassium or calcium, to a salt, Liebig further concluded that these alkalis played a crucial role in the process too.

Liebig's account of the actual sequence of acids in the pathway of photosynthesis was, however, rather vague, although he thought that initially oxalic acid might be produced from carbonic acid by the release of oxygen – given the presence of an alkaline base, light and the hypothetical "vital force", which Liebig still believed was a causal factor.[11] The latter was his interpretation of the unspecified "living cell" factor, since the vital force was thought to fade away when the living organism was damaged or destroyed. Liebig thought that, in later stages, oxalic acid might be reduced to tartaric, malic or citric acid, from which carbohydrates were then

[8] See, on Liebig, e.g. Brock (1997).

[9] The theory was first formulated in Liebig (1843), and Liebig subsequently repeated the principal idea in a number of other publications, among those his own chemistry textbook, of which there were a number of editions. On Liebig's hypothesis, see also, e.g., Florkin (1977), p. 147; Stiles (1925), p. 194, Schroeder (1917), pp. 2–3, and Rabinowitch (1945), p. 255.

[10] Liebig (1843), pp. 61–62.

[11] See, e.g., Schroeder (1917), p. 2. Although, in many instances, Liebig rejected the practice of using a vital force as an explanatory factor, he still acknowledged that there were some phenomena that could not be explained without this force. On Liebig's position between reductionism and vitalism, see, e.g., Lipman (1967), or, more recently, Hall (1980). See also the discussion of the topic in Caneva (1993).

formed, thereby releasing additional oxygen. Thus, Liebig postulated a stepwise path from carbonic acid to carbohydrates via compounds that became increasingly poor in oxygen and rich in hydrogen. Empirical evidence was taken from the fact that, in the presence of alkali and at high temperatures, the decomposition of oxalic, tartaric and citric acids to carbon dioxide had been observed in the test tube by the French chemist Joseph Louis Gay-Lussac; with this finding as a starting point, Liebig considered it entirely feasible that the reverse reaction could take place in plant cells.[12]

Liebig was also rather imprecise when it came to postulating how this sequence of acids in plants might occur. He skipped the question of possible sources of hydrogen for the reduction and totally ignored all the other aspects of the process; he failed to propose that chlorophyll and light might have any functions, nor did he discuss how carbohydrates were formed from organic acids. This is entirely understandable, given Liebig's poor knowledge of reduction processes in general and of the chemical nature of both chlorophyll and carbohydrates in particular.[13] Surprisingly, however, in view of its many shortcomings, the principal idea of his model – that organic acids were the intermediates in the gradual reduction of carbon dioxide to carbohydrates – was still being debated in the 1920s, even though both the vital force and alkalis had by then been abandoned as possible causally relevant factors. The main points in favour of Liebig's model were: first, that one could construct a stoichiometrically plausible pathway from carbon dioxide to carbohydrates through the stages of various organic acids; and, second, that it had been observed that organic acids could be found in abundance in all parts of the plant, so that their presence had to be accounted for. It was clear that they were synthesised by the plants themselves, and since these acids could indeed be considered to be the reduction products of carbon dioxide, Liebig's original proposal did not go completely out of favour until well into the twentieth century.

4.2 Adolf von Baeyer and the Formaldehyde Model

The formaldehyde model was first advanced in 1870 by the German organic chemist Adolf von Baeyer; and even far into the 1930s, as we shall see in later chapters of this book, parts of his hypothesis were still regarded as one of the most promising candidates for a photosynthesis model. In essence, Baeyer's model comprised the assumption that the first reduction product of photosynthetic assimilation, which resulted from the photolysis of carbon dioxide in the presence of water, light and chlorophyll, was formaldehyde, and that oxygen was released during this process.

It is worth taking a quick look at Baeyer's general preoccupations at this time. He is, of course, particularly renowned for his research on the plant dye indigo: Baeyer successfully synthesised this important dye in the test tube in 1880, and by 1883 he had completely elucidated the molecule's structure. (Baeyer was awarded the 1905 Nobel Prize in Chemistry, in part because of these achievements.)

[12] Liebig (1843), p. 63.

[13] However, this lack of detail is also indicative of Liebig's approach in general; he was not afraid of formulating sweeping hypotheses on the course of metabolism, even though he was largely ignorant of plant or animal physiology in general. See, e.g., Werner (2001) and Werner & Holmes (2002) for an analysis of the dispute between Liebig and Matthias Schleiden and Hugo von Mohl.

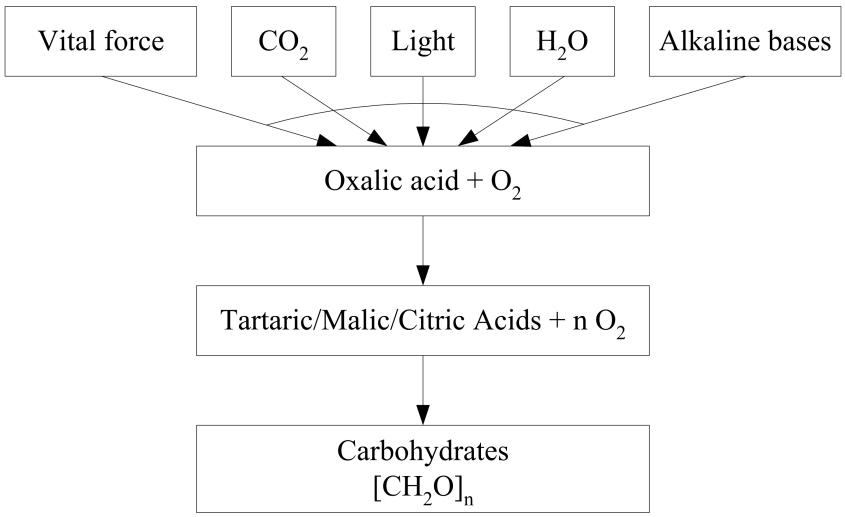

Figure I.3: The different processes involved in photosynthesis according to the organic acid hypothesis, which was originally proposed by Liebig (1843). The precise sequence of organic acids was unclear.

However, around 1870, Baeyer was more interested in condensation reactions, and he achieved a major breakthrough in 1872 when he succeeded in carrying out the polycondensation of phenol and formaldehyde. Formaldehyde, a small organic molecule, was discovered in 1855 by the Russian chemist Alexander Mikhailovich Butlerov (although it was conclusively identified by August Wilhelm von Hofmann in 1867) and it had since become a product of central interest in the field of organic chemistry.

We can safely assume, given the focus of his research at the time, that Baeyer was familiar with Butlerov's work. Thus, it is not entirely surprising to find that Baeyer based his photosynthesis model on empirical evidence that Butlerov presented in 1861: on heating trioxymethylene, a condensation product of formaldehyde which today is known as 1,3,5-trioxane, in an alkaline medium, a viscous fluid, which seemed to have some of the properties of sugar, was produced.[14] Baeyer took this as the starting point for his theory of carbohydrate synthesis in living plants:

> The general assumption in regard to the formation of sugar and related bodies in the plant is that, under the action of light, carbon dioxide is gradually reduced in the green parts [of a plant] and by subsequent synthesis is converted into sugar. [...] Butlerov's discovery provides the key [to the alternative assumption that sugar is formed directly from carbon dioxide], and it is indeed surprising that it has up to now been so little utilised by plant physiologists. The similarity that exists between the blood pigment and the chlorophyll has often been referred to; it is also probable that chlorophyll as well as haemoglobin binds carbon monoxide. Now, when sunlight strikes the chlorophyll, which is surrounded by CO_2, the carbon dioxide appears to

[14] See Butlerov (1861); the episode is also discussed in Stiles (1925), p. 194; Florkin (1977), p. 147; and Rabinowitch (1945), p. 255.

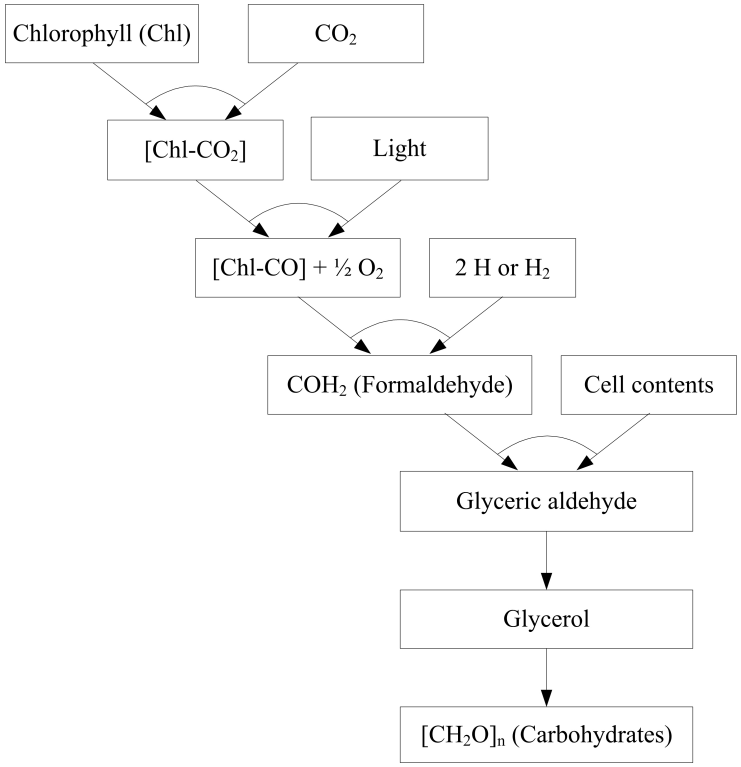

Figure I.4: The processes involved in photosynthesis according to Baeyer's formaldehyde model (1870).

> undergo the same dissociation as at higher temperatures: oxygen escapes and carbon monoxide remains bound to the chlorophyll. The simplest reduction of carbon monoxide is to the aldehyde of formic acid – it only needs to take up hydrogen, $CO + H_2 = COH_2$.
>
> Under the influence of the contents of the cells, as well as through the alkalines, this aldehyde is then converted into sugar. [...] Glycerol could, in addition, be formed by the condensation of three molecules and the subsequent reduction of the thus formed glyceric aldehyde.[15]

According to this proposition, the carbon reduction in photosynthesis consisted of several processes, which are reconstructed in figure I.4 (p. 34). First, carbon dioxide binds to the chlorophyll, which is shown as [Chl-CO_2] in the figure; in this state and under the influence of light the carbon dioxide is reduced to carbon monoxide, upon which oxygen escapes. Baeyer justified the assumption of this step by referring to the structural similarity between chlorophyll and haemoglobin; since the latter was known to bind carbon dioxide, it was reasonable to assume that chlorophyll could do so as well.

[15] Quoted in Stiles (1925), p. 194; also in Florkin (1977), pp. 147–148. Translation provided by Jörgensen and Stiles (1917), with minor changes introduced by the author. For the German original, see Baeyer (1870), pp. 67–68.

The carbon monoxide is then reduced further to formaldehyde by the bonding of either molecular hydrogen or two atoms of hydrogen from other sources (which were not specified). Thus, in contrast to the conceptualisation of the process in the organic acid hypothesis, the actual reduction of carbon dioxide to the final oxidation state (which was instantiated, for example, in formaldehyde) was assumed by Baeyer to occur without the presence of any intermediates. In subsequent reactions, the formaldehyde was then thought to produce carbohydrates – a process that was presumably promoted by the contents of the cell, the influence of which was unknown. For example, Baeyer hypothesised that the first sugar product might still be associated with the components of the cell, and that it would only later be released as sucrose, starch or cellulose.[16]

However, before Baeyer presented his own point of view, he summarily dismissed the organic acid hypothesis:

> The intermediate steps [of the gradual reduction process] have been sought in the organic acids – formic acid, oxalic acid, tartaric acid, etc. – which can be regarded as the reduction products of carbon dioxide. According to this opinion, at those times when the green parts of the plant are most strongly subjected to the action of the sun's rays, a strong accumulation of acids should take place, and these should then gradually give way to sugar. As far as I know, this has never been observed, and when it is remembered that in the plant sugars and their anhydrides are found under all circumstances, whereas the presence of acids varies according to the type of plant, the particular part and its age, then the opinion already often put forward, that the sugar is formed directly from the carbon dioxide, increases in probability.[17]

Hence, Baeyer's main objection to the organic acid hypothesis was that one of its (conjectured) empirical consequences, namely the accumulation of intermediate products at times of strong photosynthetic action, had, as yet, not been observed. Furthermore, Baeyer pointed out that the acid content of a plant was strongly dependent on parameters that were probably not connected to photosynthesis – such as the species, the part of the plant, the time of year, and so on – which did not tie in with the assumption that these acids were the intermediates of the general photosynthesis pathway. Then, having raised these two (rather unconvincing) objections, Baeyer immediately went on to present his own alternative model, without making any more references to the weak points of his predecessor's attempt. Rather, Baeyer stressed that he was able to propose a much easier and more direct pathway than Liebig had done: "Indeed, it would be difficult to attain the goal so easily through a gradual synthesis following the other theory!"[18] His proposal seemed pretty straightforward:

$$\begin{aligned} \mathrm{CO} + 2\mathrm{H} &\longrightarrow \mathrm{COH_2}(= \mathit{formaldehyde}) \\ \mathrm{n[COH_2]} &\longrightarrow [\mathrm{CH_2O}]_n(= \mathit{carbohydrates}) \end{aligned} \tag{I.2}$$

[16] Baeyer (1870), p. 68.

[17] Quoted in Stiles (1925), p. 194; also in Florkin (1977), pp. 147–148. Translation provided by Jörgensen and Stiles (1917). For the original German text, see Baeyer (1870), pp. 67–68.

[18] Baeyer (1870), p. 68.

Put into prose: if carbon monoxide is formed, you only need to add two atoms of hydrogen to arrive at formaldehyde. The latter is already very close to the basic unit of carbohydrates (which is $[CH_2O]$), so to form carbohydrates the formaldehyde only needs to be slightly rearranged and its units multiplied (which takes place during the condensation reactions); finally the resulting glyceric aldehyde had to be transformed into a sugar – although Baeyer never explicitly discussed this additional complication.

4.3 Testing and Modification

If Liebig's model had been instrumental in getting chemists to take an interest in photosynthesis, then Baeyer's contribution undoubtedly sparked off a lively discussion. The main difference between the two approaches was the concept of how the reduction of carbon dioxide was achieved: Baeyer believed that only one step was needed to arrive at carbon dioxide reduction (since the oxygen was removed all at once), whereas Liebig assumed that the process consisted of many small steps (which involved several instances of oxygen removal). What followed was a period of intense experimental work that put both models to the test. Baeyer's theory in particular was eagerly taken up by his contemporaries and succeeding generations of scientists, many of whom regarded his model as the first experimentally supported proposal to explain carbohydrate synthesis, inside and outside the living plant. The organic acid model also continued to be debated: scientists tried to find out more about the conversion of one acid into another, about the function of acids in plants and about a possible pathway of carbohydrate formation.

Much can be learned by taking a closer look at the way the formaldehyde model was received, since, even though scientists were enthusiastic about it, they did not find it entirely satisfying. All the causal hypotheses that Baeyer had included in his model were experimentally tested as scientists searched for positive evidence for the postulated causal relationships. Many pieces of evidence were subsequently discovered that seemed to support the model. For example, chlorophyll was reported to form complexes with carbonic acid *in vitro*. Furthermore, it was found that formaldehyde could, in fact, be produced by the reduction of carbon dioxide by magnesium or by the action of a silent electric discharge. The formation of formaldehyde was also observed in systems containing chlorophyll, water and oxygen. From these results it was understood that the substances in the system did act as causally relevant factors to the formation of formaldehyde – just as Baeyer had postulated.[19] A triumphant moment in the development of the formaldehyde theory came in the 1890s, when one of Baeyer's former students, the German organic chemist Emil Fischer, succeeded in demonstrating that formaldehyde was, indeed, a possible starting point for the synthesis of the two hexoses, which were thought to be among the major end products of photosynthesis (*d*-glucose and *d*-fructose). In addition, one of Fischer's suggested pathways included glyceric aldehyde as an intermediate, the possible

[19] See Florkin (1977), pp. 148–149, and Stiles (1925), pp. 194–201, for a more detailed discussion of examples.

existence of which Baeyer had already hypothesised.[20] At the same time Fischer demonstrated that glycolic aldehyde, which can also be derived from formaldehyde, could also be a possible imtermediate.[21] It was believed that these findings strongly corroborated the formaldehyde hypothesis. Not only were carbon dioxide, water (and possibly chlorophyll) causally relevant to the formation of formaldehyde; Fischer had now shown that formaldehyde was, in turn, causally relevant to the formation of the end products of photosynthesis.

However, other aspects of the model were being challenged. By the beginning of the twentieth century, for example, evidence had accumulated that carbonic acid could be reduced directly to formaldehyde, without passing through the stage of carbon monoxide, the involvement of which thus appeared more and more improbable.[22] Other studies, above all by the English physiologist Francis L. Usher and his collaborator J. L. Priestley, seemed to demonstrate that, parallel to the formation of formaldehyde, hydrogen peroxide was also formed; it was thought that the hydrogen peroxide was then removed by the enzyme catalase.[23] The idea that peroxides were somehow involved inspired many scientists to continue trying to model the path from formaldehyde to carbohydrates (see section 5, pp. 42ff. of this chapter). And, finally, whereas Baeyer had not dared to advance any hypothesis as to the origin of the hydrogen that took part in the reduction process, in 1906 the German chemist Walter Löb proposed that the hydrogen might originate from the cleavage of water.[24]

Thus, although over time it came to be believed that many of the causal hypotheses in Baeyer's formaldehyde model needed to be revised, its main assumptions were held to be essentially correct for a very long time, notably that:

(i) chlorophyll forms a complex with carbon dioxide, in which the latter is reduced through the action of light;

(ii) formaldehyde is the first stable reduction product;

(iii) carbohydrates are formed by multiplying the resulting units (formaldehyde or its immediate derivatives).

New data became available and new theoretical insights were developed, which prompted scientists to adapt the propositions to the newly available knowledge. Note, however, that these scientists did not intend to disprove (that is, "falsify") the model. Rather, they were constructively modifying it – to the extent that one could describe them as entirely new models, even though traces of the earlier models were

[20] See Schroeder (1917), p. 20 and p. 67 for a discussion of these findings. Fischer explicitly related his study of sugar synthesis to Baeyer's formaldehyde model. A comprehensive summary of Fischer's achievements on sugar synthesis, written in 1890, was reprinted as Fischer (1909*a*).

[21] See Fischer (1909*b*), pp. 31–32, and Schroeder (1917), p. 20 and pp. 67–68.

[22] For the pertinent references, see, e.g., Schroeder (1917), pp. 78–79.

[23] See, e.g., Usher & Priestley (1906).

[24] Löb was by no means the first to propose that water might be the source of photosynthetic oxygen (or, one source among several). A much earlier proponent was, e.g., the well-known French chemist Marcelin Berthelot, who, incidentally, also played with the thought that formic acid or other members of the formyl group might be the first product of assimilation. The water hypothesis had always been around since then.

still discernible. The astonishing fact is that, in the case of the formaldehyde model, these modifications proved to be fruitfully possible for a period of almost sixty years, which again was taken to support the assumption that the formaldehyde model was on the right track – despite the fact that it proved impossible to confirm that formaldehyde even existed in the pertinent system.

4.4 Hierarchy of Goals and Options

It is worth pausing for a moment to reflect on the state of affairs from a systematical point of view. Figure I.5 (p. 39) captures the situation up to this point in terms of the goals pursued and the collective action undertaken. The superordinate goal (the top box in the flowchart) is clearly defined: to find the accurate and adequate causal graph for photosynthesis; while the goal of the collective of chemists was narrowed down to finding the biochemical pathway, from raw materials to end products (second box from the top). Arrows leading from one level to the other denote the order of hierarchy, and at the same time indicate that the higher level is relevant to the lower levels (potential causal relationships from bottom to top have been neglected for the moment). The two central problems are repeated in the third box (the reduction of carbon dioxide; the synthesis of carbohydrates); then we arrive at the two alternative models, developed by Liebig and Baeyer, both of which are attempts to provide a solution. Liebig presented a variant in which the carbon was reduced gradually in a process that consisted of a number of small steps and which assumed that carbohydrates were formed via organic acids (Alternative A in the figure); while thirty years later Baeyer proposed that the reduction occurred in one single step and that carbohydrates were formed via formaldehyde (Alternative B). These two alternatives then were being tested and, if considered appropriate, modified; and while the formaldehyde model was favoured by most of the members of the collective, it is important to note that the organic acid alternative was still being thoroughly explored. This phase of testing and modification can be interpreted as being the collective's attempts to adapt the models in order to find a better "fit" for its goals.

However, it soon transpired that the experimental testing of the suggested causally relevant factors yielded unsatisfactory results, with the findings remaining contradictory, so that only a few well-targeted modifications were possible. Consequently, there was a change of strategy, at least in parts of the collective: instead of trying to test and modify the causal hypotheses of one or the other model, the scientists started looking for variants that recombined what were considered to be the respective strengths of the alternatives. There was, for example, a significant number of chemists who thought that Baeyer's idea of carbohydrate formation via formaldehyde was a promising approach, although they were not so convinced by Baeyer's theory that the reduction of the carbon moiety occurred in one single step; they preferred to look for another solution, possibly incorporating a Liebig-like mechanism via organic acids. This is schematically represented in figure I.6 (p. 40): a new goal emerged that required the scientists to find a model that included: (1) a path in which carbon dioxide was reduced via organic acids to formaldehyde; and (2) the synthesis of carbohydrates from formaldehyde.

In the following decades, four main alternatives were put forward: the formic acid model, proposed in 1877 by Emil Erlenmeyer; the organic acid-formaldehyde

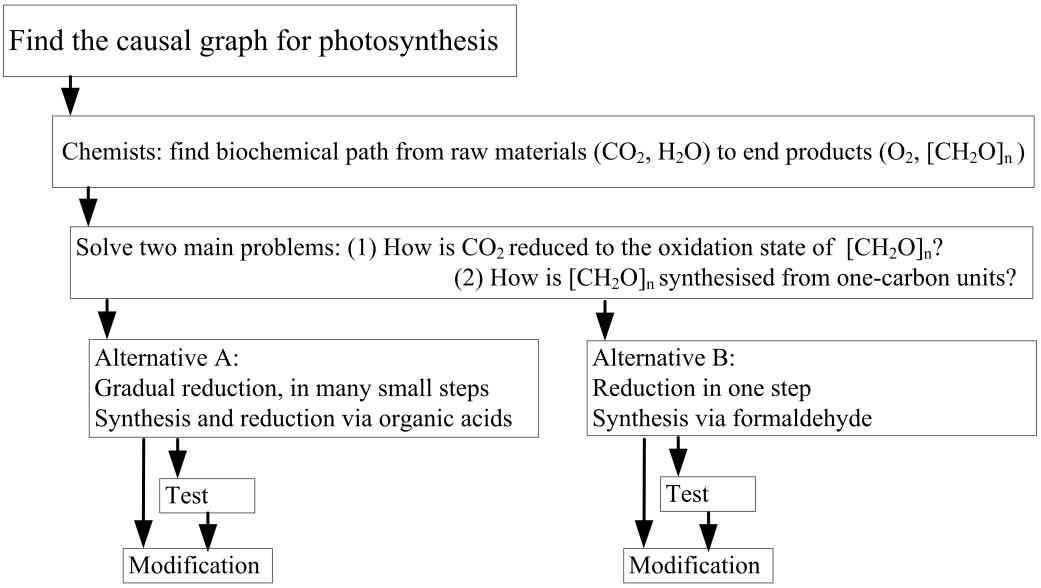

Figure I.5: Hierarchy of goals and options after the publication of the formaldehyde model.

model, advanced in 1913 by Emil Baur; the water cleavage model proposed by Georg Bredig in 1914 and the chlorophyll complex model of 1918, developed by Richard Willstätter and Arthur Stoll.[25] This list of well-known names, which includes the most eminent figures of the time, makes it clear that the elucidation of photosynthesis had developed into a problem that was being taken seriously, and that many of the period's best chemists were intent on solving it.

However, before these alternatives can be presented, we need to clarify the status of the models and their evidence. Photosynthesis researchers of later periods were rather quick to dismiss the nineteenth-century approaches as being based on nothing but speculation; the formaldehyde model, in particular, suffered not only from a lack of positive evidence but also from a wealth of negative findings. If formaldehyde really were a central intermediate, why had nobody been able to detect it in substantial amounts in plant cells? And why did the constant failures not prompt those involved to discard the model as an unproven dead end? If ever a model was empirically "falsified", the formaldehyde model was.

4.5 The Meaning of Negative Results

Scientists working around 1900 obviously disagreed with this statement. Although many were rather disturbed by the failure to detect substantial amounts of formaldehyde in plants, this circumstance did not render the model a disaster in their eyes. Some tried extremely hard to obtain formaldehyde: they not only meticulously scrutinised the ashes of plants or submitted the leaves to as many different solvents as possible; they also attempted to "feed" plants with formaldehyde via the atmo-

[25] The titles of these models were not used in the discussion on photosynthesis research in the nineteenth century but have been given by the author to facilitate the analysis of this chapter.

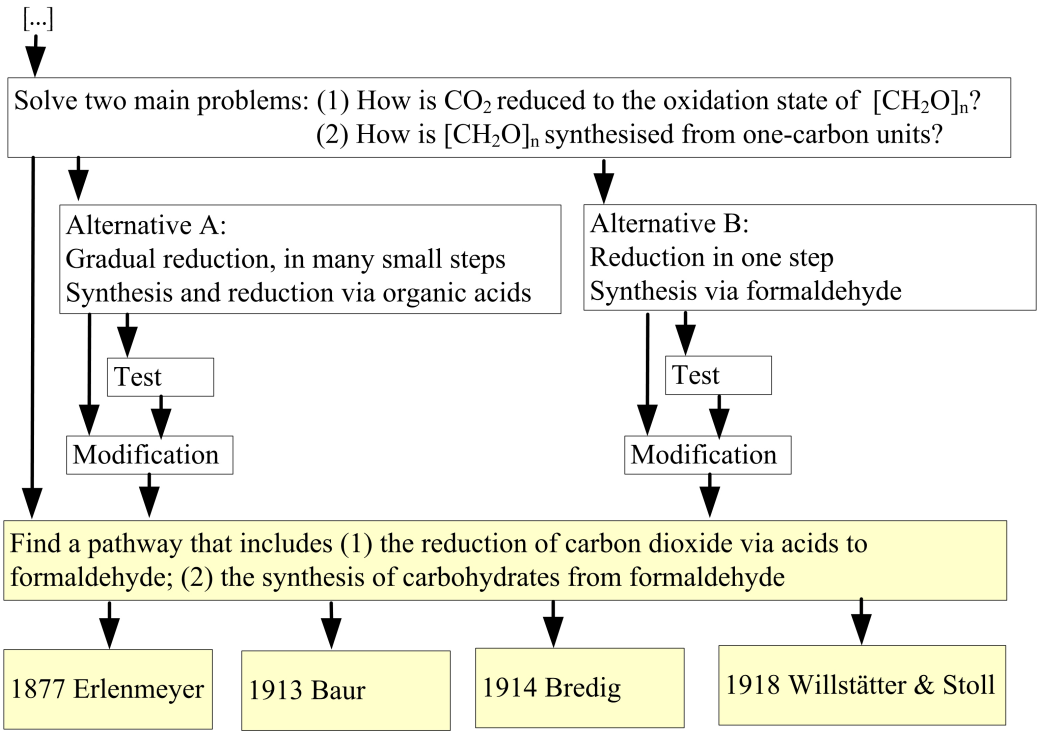

Figure I.6: New goal: to find a model that preserves the strengths, but not the weaknesses, of the two main alternatives.

sphere or an aqueous medium. The results were always negative. No one was able to demonstrate that formaldehyde occurred in plants or to detect that, in the feeding experiments, formaldehyde had a stimulating effect. Yet the scientists did not lose their faith in the model. They either refined their techniques and tried again or they attempted to meet the difficulties by explaining their failures – for example, it was soon agreed that formaldehyde had, in any case, to be processed very swiftly by plants because it was such a strong cell poison.[26] The chemist Walter Löb (mentioned earlier) even suggested that formaldehyde was perhaps never actually released as such; he surmised instead that the formaldehyde's constituents (C, OH_2) immediately condensated to sugar.[27]

This firmly held belief in the causal relevance of formaldehyde in photosynthetic carbon dioxide reduction appears at first to be entirely irrational. Knowing that the model eventually proved false, one wonders whether the scientists of the time should not have realised earlier that they were on the wrong track. Their behaviour appears to defy all principles of scientific reasoning. Even though philosophers of science have conceded that theories are, in general, extremely hard to falsify, single hypotheses should pose fewer difficulties. And if a hypothesis is falsified,

[26] See, e.g., Schroeder (1917), pp. 8–12. Manning (1938), p. 122, underlined this point, stating that, even if photosynthesis was running at a maximum rate, formaldehyde "concentrations higher than a few hundredths of a per cent are distinctly toxic".

[27] Löb (1906).

as the assumption concerning the central derivative of the formaldehyde model repeatedly was, one would expect, as an inevitable consequence, that this model would have been dropped in favour of alternatives (the organic acid model and its equally numerous variants were the obvious options).

It is tempting to assign this reluctance to abandon the formaldehyde model to psychological immobility, to the high esteem in which Baeyer was held, or even to a lack of critical thinking on the part of these scientists. However, I would like to argue that, far from using a flawed methodology, Baeyer was following good scientific practice in proposing his model; and that his successors, who refused to accept the apparently constant falsification of the formaldehyde model, were, in fact, well advised to do so. Talking about "falsification" in the context of (causally) explanatory hypotheses or models reveals serious misconceptions about the nature of these hypotheses and about the models to which they belong.[28] It was mentioned earlier that the formaldehyde model comprised a complex causal hypothesis, and that this hypothesis was then experimentally tested. I shall take only one example from the array of these tests, namely the feeding experiments. One can derive from the formaldehyde model the following straightforward hypothesis: "Since formaldehyde is a key intermediate in photosynthetic assimilation, the additional supply of formaldehyde should lead to increased photosynthesis rates." This hypothesis is, of course, a causal one – the question is whether or not formaldehyde is causally relevant to the successful completion of photosynthetic assimilation – and leads to an obvious experimental test set-up: put a plant in a closed system and measure the change in the rate of photosynthesis when an ample amount of formaldehyde is provided.

Space does not permit me to go into the numerous technical difficulties of the experiments that were actually conducted – finding the balance between insignificant supplies of formaldehyde and killing the plant by providing too much of it was only one of them. Instead, let us go straight to the results, which can be displayed as follows:

situation	Plant without formaldehyde	Plant with formaldehyde
photosynthesis rate	normal	normal

The investigated variable was the rate of photosynthesis and the testing factor was an additional supply of formaldehyde. What was found was that without formaldehyde, the rate of photosynthesis remained normal, which was not surprising; yet, even with an ample supply of formaldehyde the rate did not change. This is the negative finding in question: a non-change in effect, despite the realisation of a potentially relevant factor. An obvious conclusion from this experiment would be that formaldehyde had no relevant influence on the rate of photosynthesis – hence, it was causally irrelevant to photosynthetic assimilation. However, notwithstanding the fact that this conclusion seems obvious, this would be a fallacy; and this point of view was clearly shared by the scientists working in the nineteenth century.

[28] See Nickelsen & Graßhoff (submitted) for more details on this aspect of model-building practice.

It is an uncomfortable but inescapable feature of causal reasoning that it is impossible to infer the *causal irrelevance* of any factor directly. If the result of an experiment is negative – in the sense that the situations with or without the testing factor do not differ in outcome – then the following conclusions are possible:

(i) Formaldehyde is, indeed, causally irrelevant.

(ii) The detection method is flawed or inappropriate.

(iii) Formaldehyde is causally relevant, but at least one necessary factor of the pertinent sufficient bundle of factors was not realised.

The last condition is the decisive one. Even if all the aspects of the experimentation were correctly designed, set-up and carried out, one cannot conclude from an indifferent result, not even from a consistently indifferent one, that the respective test factor is causally irrelevant. It is always possible that the pertinent bundle of factors to which the testing factor belongs was not completely realised in the experiment – simply because it is not known what most of these factors are.

his restriction, however, does not render the causally relevant inferences unreliable. The homogeneity condition as defined in the theory by Graßhoff, May and Baumgartner requires that the test situations of a difference test are appropriately similar in so far as (a) all necessary co-factors are present, so that the causal relevance of the testing factor would be discerned; (b) the effect is not brought about by alternative causes. In other words, the two situations can, in actual fact, differ in many respects, but not in terms of fully sufficient bundles. This concept of the homogeneity condition is sufficient for a *causal relevance* inference to be made, but insufficient for a *causal irrelevance* inference to be made. The latter would require a much stronger homogeneity condition – but this would result in hardly anybody being able to meet it. Hence, it is impossible to falsify causally explanatory models from the negative (that is, unchanging) results of a difference test.

All one can hope to do is to establish positive causal relationships; and if these render an alternative that is explanatorily more powerful, then it makes sense to follow this new modelling option and drop the other. But around 1900 (and for many years to come) no other more convincing alternative lay on the horizon; however – and this is important – there were excellent arguments for believing in the principal causal relevance of formaldehyde for the synthesis of glucose: namely Fischer's *in vitro* experiments. As long as no alternative path had been established to occur in plants, this was the best option at hand.

5 Reconciling the Approaches

5.1 The Formic Acid Model

One of the attempts to integrate the advantages of the earlier models by Liebig and Baeyer, while at the same trying to avoid their shortcomings, was the assumption that the first reduction product was formic acid, which scientists believed could be further reduced to formaldehyde – the supposed precursor of carbohydrate formation.

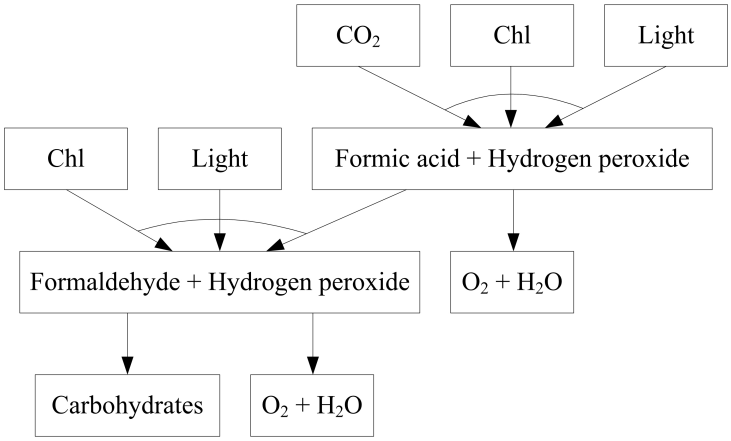

Figure I.7: The processes involved in photosynthesis according to the formic acid hypothesis, which was proposed by Erlenmeyer (1877).

The earliest proponent of this point of view was the German chemist Emil Erlenmeyer, who suggested, in 1877, that, in the process of photosynthesis, carbonic acid was first reduced to formic acid; this would yield hydrogen peroxide as a by-product, which would then be immediately decomposed into water and molecular oxygen.[29] A graphical reconstruction is given in figure I.7 (p. 43). This proposal was mainly based on experiments that Erlenmeyer had carried out with glycolic and lactic acids: both these acids were readily decomposed following the pattern described above, so that Erlenmeyer believed he could postulate that carbonic acid reacted in the same way (although he had not been able to test it in the laboratory). As Erlenmeyer wrote, he was convinced that, in view of the ready decomposition of hydrogen peroxide, this path was the most obvious way to explain the liberation of free oxygen in photosynthesis. In subsequent steps, Erlenmeyer assumed that formic acid would, under the influence of light and chlorophyll, be further reduced to formaldehyde and then polymerise to form carbohydrates.[30] And even though, along with formaldehyde, the presence of formic acid and hydrogen peroxide was also never detected in the green parts of plants to any substantial extent, scientists continued to debate this model until well into the 1930s.[31]

5.2 The Organic Acid-Formaldehyde Hypothesis

An alternative hybrid model that tried to combine the advantages of Liebig's and Baeyer's approaches was proposed in 1913 by the Swiss physical chemist Emil Baur.[32] A graphical reconstruction is given in figure I.8 (p. 44). In line with Liebig's hypothesis, Baur argued that it was highly improbable that the reduction of carbon dioxide or, rather, carbonic acid, was accomplished in one single step, as Baeyer had postulated. Considering the respective oxidation states of the carbon atom,

[29] Erlenmeyer (1877).
[30] Erlenmeyer (1877), p. 634.
[31] See Stiles (1925), p. 199.
[32] Emil Baur is not to be confused with the German geneticist *Erwin* Baur, who made important contributions to the synthetic theory of evolution in the 1920s.

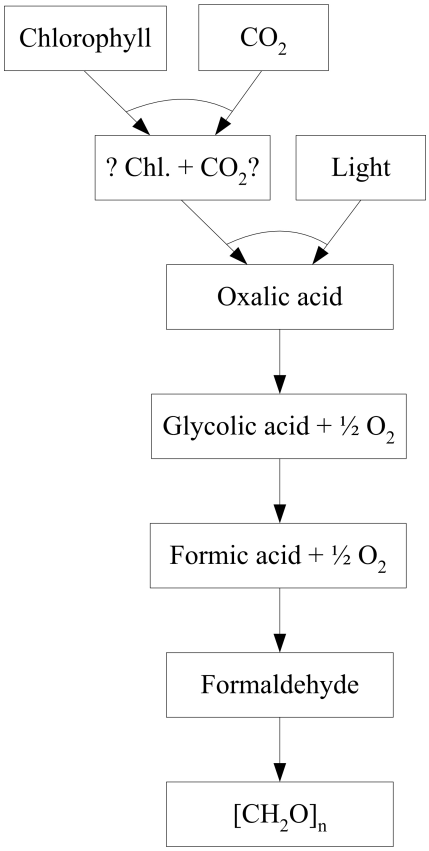

Figure I.8: The processes involved in photosynthesis according to the organic acid hypothesis, which was advanced by Baur (1913).

Baur thought that several potential intermediates might be formed on the path from carbon dioxide to carbohydrates; and since chemical processes almost always include the formation of intermediates, as Baur pointed out, he preferred to assume that they did, in fact, occur.[33]

Baur was convinced that oxalic acid was the first product of photosynthesis, and that it was produced after the carbon dioxide had interacted with the pigment, which then absorbed and utilised the light energy – two different processes, neither of which Baur discussed in any detail.[34] In the later stages of the gradual reduction of carbonic acid, which, most probably, involved the formation of glycolic and formic acids, oxygen would be released. And, although Baur found it highly improbable that formaldehyde was the first reduction product, he nevertheless believed that the final stage of photosynthesis in which carbohydrates are formed was reached via formaldehyde.

[33]See, e.g., Baur (1913), p. 474. To substantiate this point, Baur also cited H. Euler, Pflanzenchemie, 1909, III. Teil (Part), pp. 183 and 266; as well as his own monograph, *Cosmografia Chimica*, Milan, 1908, p. 207.

[34]Baur (1913), p. 475.

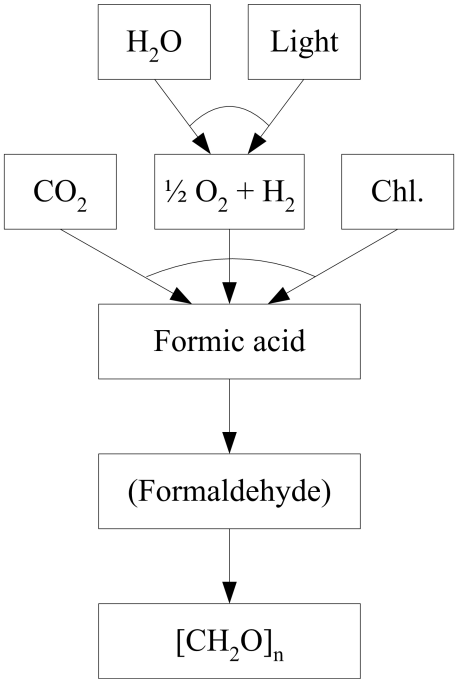

Figure I.9: The processes involved in photosynthesis according to the water-cleavage hypothesis, which was proposed by Bredig (1914) and Hofmann & Schumpelt (1916). While Bredig was doubtful concerning the role of formaldehyde, it was reinserted by his successors; therefore the factor is put in brackets.

5.3 The Water Cleavage Model

A third hybrid variant was proposed by the German physical chemist Georg Bredig in 1914,[35] whose suggestion was subsequently supported by the chemists Karl August Hofmann and Karl Schumpelt.[36] A reconstruction is given in figure I.9 (p. 45). Bredig seriously doubted the validity of the formaldehyde hypothesis: apart from the fact that there was no convincing evidence that formaldehyde occurred in green plants, he pointed out that "the formaldehyde, in any event, is the one reduction product of carbonic acid, the production of which would require *the highest energy input* by light; and for this reason alone it is not very likely that nature should have chosen this detour".[37] In line with Erlenmeyer, Bredig proposed that formic acid was the first product to be formed, which was supported, he believed, by his recent finding that the salts of formic acid were, under moderate pressure, produced from the salts of carbonic acid in the presence of a surface-providing catalyst, such as palladium. This also tallied with his earlier discovery that, under the influence of surface-providing catalysts, hydrogen was removed from organic substances and transferred to other molecules.

[35] Bredig (1914*b*); see also Bredig (1914*a*) and Bredig (1915).
[36] Hofmann & Schumpelt (1916).
[37] Bredig (1914*b*), p. 363.

Bredig suggested that in nature the catalytic function could be ascribed to the chlorophyll, while the hydrogen came from water cleavage: it had been observed, after all, that, under the influence of ultraviolet light, water decomposed into an explosive mixture of molecular oxygen and hydrogen (oxyhydron gas, called *Knallgas* in German). In plants, the catalysing agent took the hydrogen from the decomposition of water and used it in the reduction of carbonic acid, whereby oxygen was released. Thus, Bredig was one of the few scientists of these decades to address explicitly the question of the origin of the reducing hydrogen equivalents; and he was one of the few scientists to consider water as a possible source of hydrogen and, at the same time, oxygen. (Walter Löb and some other contemporary chemists also cautiously held this view.) However, Bredig admitted that water might be replaced as the source of hydrogen in plants by other substances that would remove the hydrogen under the influence of sunlight. Bredig did not explain how the subsequent stages from formic acid to carbohydrates were accomplished; Hofmann and Schumpelt, however, held the view that formic acid could be further reduced to formaldehyde (even without the presence of hydrogen peroxide), which then served as a starting point for the synthesis of carbohydrates following the well-known sequence.

Although this model would seem at first to be of great interest, particularly in view of later developments and today's assumption that water cleavage is, in actual fact, the source of oxygen in photosynthesis, Bredig's proposal was far from popular.[38]

5.4 The Chlorophyll Complex Model

The last model that I wish to present in this context is the one advanced by Richard Willstätter and Arthur Stoll, which emerged as a result of their comprehensive 1918 monograph on the role of chlorophyll in photosynthetic assimilation.[39] A reconstruction of this model is given in figure I.10 (p. 47). From their experimental findings, Willstätter and Stoll concluded that, once carbon dioxide had found its way into the plant's green cells, the first stage of photosynthesis consisted of a dissociable binding of the gas to unknown, organic constituents of the plant cell, presumably plant proteins or amino acids. It was by this means that the concentration of carbon dioxide within the plant cells was thought to increase, so that photosynthesis operated more efficiently. (After all, the concentration of carbon dioxide in the air is rather low.) Willstätter and Stoll surmised that the carbon dioxide was probably chemically altered in the course of these events, converted either into carbonic acid or into one of the latter's derivatives. The product of this absorption process, which was considered to be a purely chemical, light-independent process, is symbolised in the figure as the [CO_2*-Cell complex], where the asterisk indicates that the original carbon dioxide was added to this complex in a modified form.

Willstätter and Stoll believed that the modified carbon compound was then passed to the chlorophyll. From their experimental findings, they postulated that an additive compound of the bicarbonate type was formed by chlorophyll and either

[38] See Czapek (1913), p. 524, for a review of this theory in the standard plant physiology textbook of the time.

[39] Willstätter & Stoll (1918).

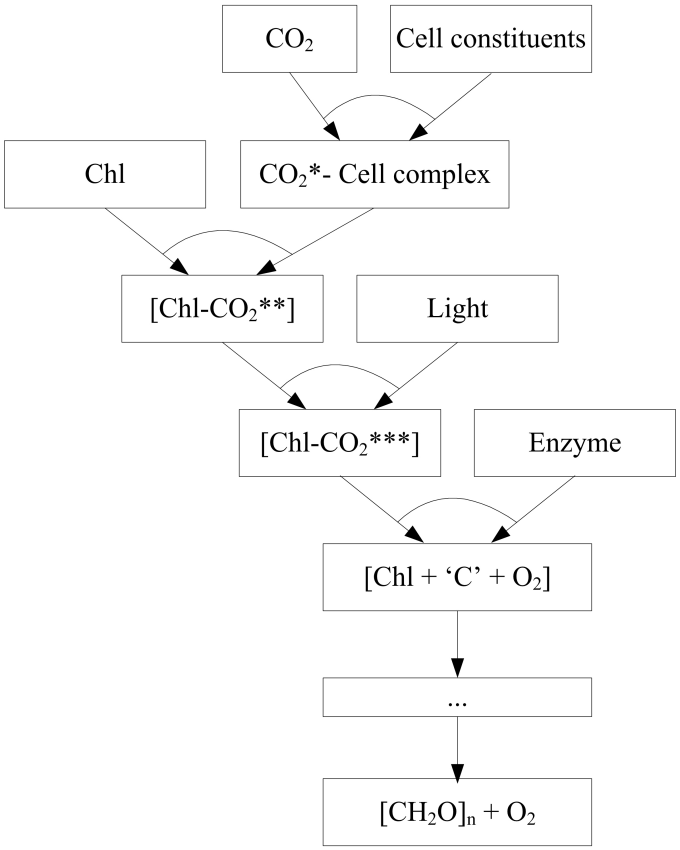

Figure I.10: The processes involved in photosynthesis as conceptualised by Willstätter and Stoll (1918).

carbonic acid or one of its derivatives. This intermediate substance is symbolised in the figure as [Chl-CO_2**], indicating that the original carbon dioxide had undergone a second conversion. The actual photochemical step of the process was believed to be the chemical rearrangement of the carbonic moiety of this intermediate, additive product into an isomer, which was higher in energy and was then reduced in the process that followed (indicated in the figure as [Chl-CO_2***]). Willstätter and Stoll assumed that this product was a kind of peroxide, most probably formylhydroperoxide.

It was thought that the further decomposition and reduction of this compound was effected by an enzyme, which Willstätter and Stoll assumed was the mysterious protoplasmic factor of photosynthesis, the involvement of which had been postulated by many of their predecessors. They surmised that, in the course of this catalysed reduction process, oxygen was released and formaldehyde synthesised, and that subsequently carbohydrates were produced via condensation reactions – we have seen this pattern in most of the models discussed so far. However, in view of the assimilatory quotient of unity, Willstätter and Stoll argued that it was highly probable that no intermediate product was released before the reduction process of the carbon had been completed, so that the stages of this reduction

process were very hard to establish.[40] In figure I.10, the result of these reactions is, therefore, symbolised only vaguely by the expression [Chl + 'C' + O_2], which is the last intermediate before the final carbohydrate stage $[CH_2O]_n$ is reached.

On the whole, and in contrast to the other scientists mentioned so far, Willstätter and Stoll considered chlorophyll to be the central factor in the whole process of photosynthesis: in addition to its capacity to absorb light and make it chemically available, chlorophyll was also assumed to be the actual site of carbon reduction, which involved the formation of an intermediate additive compound. The action of light was thought to be effective only in interaction with this additive compound; that is, the light did not act on the chlorophyll, as one might assume, but on the carbonic moiety, which was thereby converted into one of its isomers. This differed sharply from the widely held view at the time that chlorophyll only acted as a sensitiser in photosynthesis, in that it transformed rays of shorter wavelengths into more efficient rays of longer wavelengths – although it was unclear which effects these rays would then bring about.[41]

6 Features Common to the Models

By the year 1918, therefore, there existed a wide range of models to explain the mechanism of photosynthesis, and although most of them had elements in common, they also differed in many ways. However, despite their divergences in terms of content, all the models shared a number of structural features. I shall now highlight some of these features, which are typical of scientific models and their development in general.

6.1 Simplicity and Simplification

The most striking feature that all the models discussed in this chapter have in common is their simplicity. Although it was clear to all the scientists involved that the process of photosynthesis was highly complicated, none of the models included more than a very limited number of factors and reaction steps. Indeed, simplification, sometimes to the extreme, is a typical, well-known and indispensable aspect of the construction of scientific models, even though the concept is a notoriously ambiguous one. If they are to produce anything that is in the slightest way useful, scientists need to keep to the factors and reaction steps that they consider central, particularly in those situations where very little is known of the process to be modelled. I shall now briefly explain in which respects photosynthesis models were "simplified".

In the case of photosynthesis, one strategy of simplification was that the scientists restricted themselves to those factors whose causal relevance had been established beyond any doubt, although it was clear that many additional but yet unknown factors were also involved. In particular, almost all the scientists involved limited their choice of root factors to those of the previously discussed one-step model, although most of the actors did not include the structural root factor

[40] The "assimilatory quotient" is the ratio of the amount of carbon dioxide taken up to the amount of molecular oxygen evolved during photosynthesis.

[41] See, e.g., Czapek (1913), p. 614, for a review and emphatic endorsement of the "sensitiser" position.

in their models.[42] There were only two exceptions: first, Liebig, who postulated the involvement of the vital force and alkaline bases (both of which were almost immediately dropped by his successors); and, second, Willstätter and Stoll, who believed that an unknown enzyme was an additional root factor, which they assumed catalysed the final reduction steps. Scientists also limited the end products of their models to those of the one-step model, although at the time nobody could be sure that these end products really were all that came out of the process. The only argument for this assumption was that the assimilatory coefficient in most cases approached unity, which corresponded well to the assumption that only molecular oxygen and carbohydrates were produced, since the formation of, for example, proteins or fats would have yielded different coefficient values. However, one could easily have constructed scenarios that might have included the formation of a mixture of substances, which would still have produced an assimilatory coefficient of unity.

To a certain extent, a principle of parsimony also governed the (mostly implicit) assumption that the same mechanism of photosynthesis operated in all species of higher plants. None of the actors mentioned above considered it even worth their while to discuss the possibility that in the leaves of trees, for example, the process of photosynthesis might differ from the process of photosynthesis in the leaves of grass – although this was by no means self-evident, given the enormous differences between these species of plants. In his widely read textbook on photosynthesis, the plant physiologist Heinrich Schroeder believed that one could safely assume that the same mechanism was valid for all higher plants, because "given the same raw materials (CO_2 und H_2O), the same products are found, while the circumstances as well as other resources of the transformation are also the same".[43] One could object, however, that this was only partly correct: many morphological and physiological differences were known to distinguish different species of plants from each other; and although these differences were silently assumed to be irrelevant to the photosynthetic mechanism, no one could be absolutely certain that this was indeed the case. (In fact, as it later turned out, this assumption was not correct, since it is now known that there are different forms of photosynthesis.)

Resorting to assumptions like these was entirely natural. The mechanism of photosynthesis is so complicated that none of the scientists working in the nineteenth century could possibly have hoped to achieve more than a rudimentary understanding of it. In view of this situation, those scientists working in the field seemed to have agreed that it was inadvisable to make things even more difficult by taking into consideration more factors than was absolutely necessary, not to speak of possible detours or variants of the pathway. Methodologically speaking, scientists shared the working hypothesis that photosynthesis acted as a sequence of the same type of events in all higher plants; thus, if the causal relationships were established in one instantiation, the same should be true of all others. These construction assumptions are an essential part of the modelling process: for the sake of constructing the model, scientists consciously assumed that the process had certain properties, even though they knew (or, at least, strongly

[42] "Root factors" are the starting nodes of a causal graph.
[43] Schroeder (1917), p. 36.

suspected) that this was not actually the case. It would have been disastrous had the scientists working around 1900 attempted to model the process in all its variants and complexities: total defeat would surely have been the outcome.

6.2 Incomplete Empirical Support

The second common feature that I wish to highlight is the fact that all the photosynthesis models were underdetermined by empirical data to the highest degree. In stark contrast to the conservative attitude towards introducing new root and end factors to the process, the scientists of the period under study apparently did not mind postulating completely new *intermediate* factors and causal relationships: this is true, for example, not only of all the assumptions about the products of the gradual or immediate reduction of carbon dioxide but also of the formation of a complex made of chlorophyll and either carbon dioxide (Baeyer) or an unknown derivative of carbonic acid (Willstätter and Stoll). None of the actors seemed too concerned about invoking either unheard-of reaction steps and mechanisms or those which were not yet satisfactorily explained. Still, none of these models was discarded by the scientific community on the grounds of incomplete experimental foundation, which seems to suggest that underdetermination was not only common but also accepted practice at the time.

Take the formaldehyde model as an example. Baeyer's central assumption – that formaldehyde was formed in living leaves and that the formaldehyde molecules subsequently combined to larger units under the influence of the cell's components – lacked any kind of experimental support taken from investigations carried out on plants or plant cells. The hypothesis that carbon dioxide formed a complex binding with chlorophyll was exclusively based on the observation that chlorophyll was structurally similar to haemoglobin; and since the latter was known to bind carbon dioxide, the former was assumed to do the same. All inherent differences were silently considered irrelevant. Yet both principal assumptions – the formation of a chlorophyll complex and the synthesis of carbohydrates via formaldehyde – were integrated into almost all the subsequent photosynthesis models, including, for example, those of Erlenmeyer and Baur, who held distinctively different views on the first stages of the reduction process but who nevertheless concurred with Baeyer on the principle of synthesis.

Even when empirical data were presented, scientists tended to back up their models with experimental evidence that had been gathered from the observation of artificial systems, which contrasted starkly with the conditions in real plants. The formaldehyde hypothesis, for example, was exclusively based on observations carried out under quite extreme conditions – not only in its original form but also in its many variants in the ensuing decades. Likewise, the evidence for the formic acid hypothesis was solely based on scientists' observations of test tube experiments, which were then transferred to specific life processes. This type of reasoning was omnipresent: scientists used evidence that had not been taken from observations they had made of the living organism itself but from the chemical reactions that had occurred outside it, either to explain single steps of the process or, more usually, the whole sequence of events. Willstätter and Stoll were, to some extent, exceptions to this rule, since they actually measured the gas exchanges of living material. However, when it came down to the mechanism of the process

itself, Willstätter and Stoll also used empirical data that were unrelated to their physiological observations.

6.3 Selected Focus and Modules

Closely related to the principle of simplicity is the third observation to which I would like to draw attention: all the models, to a greater or lesser extent, were inherently inconsistent in their level of detail and explanatory scope. In the models the scientists all treated at length a specific aspect or a particular process of photosynthesis, while totally neglecting, other also relevant aspects. Although specific processes were spelled out in detail, other sequences of events were merely summarised.

Take, for instance, Liebig's model, the crux of which was the assumption that the formation of carbohydrates occurred in a series of intermediate stages, each of which included the formation of organic acids that became increasingly poor in oxygen and rich in hydrogen. Liebig failed, however, to explain how these acids were thought to be converted into carbohydrates. This neither means that he considered this reaction step unimportant, nor that he thought that this step had been adequately treated in the model. The synthesis of carbohydrates was, most probably, ignored, because it lay outside Liebig's focus of investigation. Baeyer's focus was the introduction of formaldehyde as the central intermediate on the path of carbon from carbon dioxide to carbohydrates. He also hypothesised about the complex of chlorophyll and carbon monoxide, yet remained silent on all the details. Finally, Willstätter and Stoll concentrated on the first stages of photosynthesis, which involved the function of chlorophyll, although when it came to the actual carbon reduction they vaguely postulated that an intermediate substance, which was peroxide in nature, was formed, and that it would eventually pass through the stage of formaldehyde and polymerise, as in Baeyer's model.

To some extent, incompleteness is a consequence of simplification: if one chooses to deal only with the central aspects of a problem, one will inevitably present an incomplete account of it. One also has to consider that models are always constructed to cover the phenomenon in question from a specific perspective and in view of specific applications. However, in this particular case the application and intended scope of the models were fairly similar to each other, yet the scientists still presented widely divergent accounts. And it is striking that the chosen focus of research corresponded so closely to the authors' general knowledge and competencies. Willstätter and Stoll were experts in the field of chlorophyll research, while they only a limited knowledge of carbohydrate formation; it was understandable then that their model should be more detailed in the former respect than in the latter.

7 Model-Building Heuristics

In summing up the observations made so far, it would seem that the process of modelling photosynthesis had taken an unusual path: even the most promising models dealt with a highly simplified notion of photosynthesis; all the models were strikingly incomplete and focused on selected processes only; and, finally, none of the available models was more than partially supported by experimental evidence,

while the available data were mostly gathered from artificial systems, which had little in common with the living plant. On closer consideration, however, these observations are neither unusual nor should we find them surprising or disturbing. I shall now discuss the extent to which these observations reflect some of the basic principles of model-building heuristics.

7.1 Extending A Prototype

At the beginning of the chapter, I gave an outline of the body of generally accepted knowledge of photosynthesis, which can be regarded as a very basic, prototypic model that includes only the root factors (mainly the raw materials) and the final effects (the end products) of the process. Notwithstanding all the further developments made, the causally relevant factors contained in this one-step model were carried over into all the subsequent alternatives discussed in this chapter. The standard raw materials and end products of the process were well established and never seriously questioned.

One can interpret the photosynthesis models introduced in this chapter as extensions of this first model into different directions, through a reasoned modification process.[44] On the one hand, one could argue that Liebig's model was condensed, since the vital force was dropped as a root factor from succeeding variants. On the other hand, in his version Baur both modified (by introducing a different sequence of acids) and extended (by adding the synthesis of carbohydrates via formaldehyde) Liebig's proposal. I shall explore this modification process in more detail in the rest of this section.

7.2 The Transfer of Causal Knowledge

I shall now return to the formaldehyde model in order to explore this modification process more fully. At first sight it might appear surprising that contemporary chemists were so enthusiastic about Baeyer's proposal. As mentioned earlier, the experimental data put forward in favour of the formaldehyde model were exclusively derived from artificial systems, which contained carbon dioxide, water and, sometimes, chlorophyll.[45] It had been demonstrated that, under certain conditions, formaldehyde could be produced from these products and subsequently polymerised to carbohydrates. However, the conditions in question were usually very different from those predominant in the plant; indeed, most of the time they were extremely unfavourable to any life-sustaining process, such as temperatures of 250°C and several atmospheres of pressure. Neither Baeyer nor the other chemists seemed particularly disturbed by this fact; and even the eminent Wilhelm Pfeffer, who was the author of the standard plant physiology textbook of the time, admitted that, although it was too speculative for his taste, the formaldehyde model of photosynthesis was rather appealing.[46]

In the best explanatory models all causal relationships should be based on the findings of difference tests, which can be defined as the observation of the outcome of a set of homogenous situations that includes all the relevant factors, with the

[44] See Graßhoff (1998*b*) for a discussion of how models can be extended, condensed or modified.

[45] See Florkin (1977), pp. 148–149.

[46] See Pfeffer (1897), p. 339; in the original German text Pfeffer used the attribute *sehr ansprechend*.

exception of the test factor. If only the situation in which the factor is realised yields the effect, one can infer the causal relevance of the factor to this effect. Simple difference tests are needed to establish whether a factor is, in principle, causally relevant to an effect, while a number of "four-field tests" need to be carried out in order to learn about the interaction of the factors and their arrangements in bundles: the recently established, causally relevant factor to an effect is tested in combination with previously found factors in order to establish whether they are part of the same bundle (and therefore only act in combination with each other) or part of different bundles, that is, alternative causes of the effect.[47] However, the actual route to this ideal situation is complicated and cumbersome, and much time is needed before an explanatory model can even approximate this final stage of perfection. Scientists would be ill-advised to discard a model in its early stages because of empirical underdetermination; if they did, no model would ever reach an advanced stage.

Let me briefly recall the situation in which Baeyer launched his photosynthesis model. The only incontrovertible facts about photosynthesis had been incorporated in the one-step model outlined in the beginning of this chapter. It was a useful prototype and the established (by means of difference tests) causal relationships between the raw materials and end products had to be retained. However, it was clear that this prototype model urgently needed to be elaborated, although it was impossible to investigate the intermediate steps of the process by undertaking difference tests on the plants themselves. (More direct access only became possible much later, with the introduction, in the late 1940s, of radioactive tracer molecules in metabolic studies.) The best scientists could hope to do was to transfer the established knowledge of causal relationships from other (test-tube) situations to the photosynthesis processes in the living organism. Although Baeyer's model, and all the other photosynthesis models at the time, were constructed without any proof having been acquired from the living plant this does not imply that the models were constructed without the application of any form of evidence at all.

The evidence on which Baeyer and others based their model hypotheses was taken from the areas of their expertise, outside the plant sciences. As a chemist Baeyer was certainly qualified to judge the possibility of chemical reactions in general, and around 1870 the polycondensation reactions of formaldehyde were the focus of his research. Baeyer knew, too, that carbohydrates were structurally composed of a series of molecular units [CH_2O], which were identical to formaldehyde in terms of atomic composition, and he was also familiar with the potential intermediate steps of the required rearrangement of these molecules – steps whose occurrence was known from general chemistry. Based on this knowledge, Baeyer proposed that possible links existed between the established fragments of the photosynthetic process: causal relationships that were known from *in vitro* experiments. And then, as a working hypothesis, Baeyer assumed that the (unknown) process inside the plant should operate according to the principles that were known of the processes that occurred outside the plant. This same assumption explains how Baeyer came up with the earlier stages of his model. He assumed, for example, that carbon monoxide would initially form a complex with chlorophyll. In 1870, nobody knew

[47] See, e.g., Graßhoff & May (2001) and Baumgartner & Graßhoff (2004).

which complexes chlorophyll was able to form because chlorophyll had proven very elusive and impossible to isolate. Yet it was well-known that chlorophyll was structurally similar to haemoglobin, and that the latter easily bonded carbon monoxide. Thus Baeyer's causal link here was based on the (chemically speaking, well justified) assumption that molecules which are structurally similar (in relevant aspects) undergo similar chemical reactions. It was also known that, under certain circumstances and under the influence of strong light, carbon dioxide could be reduced to carbon monoxide. And although it was clear to everybody at the time, including, most probably, Baeyer himself, that this suggestion would not be the final solution (it needed to be tested and elaborated in subsequent studies), this did not mean that his proposal was flawed in its methodology and should thus be immediately discarded.

From the perspective of a philosophical tradition in line with Mary Hesse's work on models and analogies, Baeyer's way of thinking may be categorised as "analogical reasoning".[48] This recourse to "analogy", however, does not satisfactorily explain the methodology behind the procedure. The latter requires one to frame the situation in terms of a causal analysis: the underlying assumption was that the process under study (in this case, photosynthesis) fell into the same class of events as other, already well-known processes. The relationship of causal relevance always concerns types of events, and not only individual tokens, so that the grouping together of processes into the same class of events means that they ought to include the same causal relationships. If carbohydrates are produced from carbon dioxide and water, it is not so far-fetched to assume that this is accomplished along the lines of similar reactions occurring outside the plant. This assumption – that similar products are effected by similar causes and mechanisms – has been a well-proven and in many cases very successful heuristic strategy, even before Sir Isaac Newton formulated this piece of advice in the form of his second Rule of Reasoning. Of course, this strategy is fallible. And, of course, the conditions under which carbon dioxide reduction occurs within and outside the organism are very different. To allow for this, additional factors for modelling the processes in the cell were introduced – supported by the observation that these life processes stopped as soon as the cell was damaged. Thus, Liebig had turned to "vital forces" to explain how photosynthesis operated, while Baeyer assigned a special function, perhaps of a catalytical nature, to the material constituents of the cell. These factors were no more mysterious than the assumption that water was one of the raw materials. They filled an explanatory gap, and it was usually expected that they would be replaced by more specific factors as advances were made in the subject.

Photosynthesis was only one of the life processes that scientists were trying to address in these terms in the second half of the nineteenth century. Plant physiologists had recognised that further progress in their field of study could only be made if both chemical and physical knowledge were used to complement more phenomenological approaches that used techniques such as gas exchange measurements.[49] Chemists, too, were gradually discovering that the processes in living organisms were an additional, potentially rewarding field of research to which

[48] Cf. Hesse (1963).

[49] See, e.g., Pfeffer (1897), pp. 1–7.

they could usefully contribute.[50] At the time, the dominant research goal within functional biology was a reductionistic one: to explain biological phenomena on the basis of general physical and chemical laws. It was widely assumed that the internal processes of an organism could be explained in the same way as the external processes. The explanation of respiration as a slow combustion process was a powerful example of this way of reasoning and one that many chemists (and biochemists) tried to emulate. Transferring the causal knowledge of other domains to the internal processes of the organism was the only way of establishing causal relationships, which is why the discovery (made by Fischer) that glucose could be synthesised via formaldehyde was received as a decisive breakthrough in proving the accuracy of Baeyer's model.

7.3 The Building Block Strategy

Transferring causal knowledge from one situation to another is, to some extent, connected to a second equally widespread heuristic strategy in model building. When one examines the different photosynthesis models it is clear that most of them are not completely disparate. Rather, one finds recurrent "modules" that reappear in different combinations, such as the "formation of a chlorophyll-carbon dioxide complex", the "reducing of carbon dioxide via organic acids", the "formation of carbohydrates from formaldehyde", and so on. Translated to causal graphs, these modules frequently correspond to one "branch" of the graph (or to one section of a longer branch), that is, these modules consist of a certain sequence of factors that are no longer being investigated, but have been "grafted" onto other model trees, if one remains with the branch metaphor.

Perhaps the most striking example of this practice is the assumption that the condensation of formaldehyde to carbohydrates takes place, a postulation that was integrated as a causal hypothesis into most of the later models. Once the causally relevant factors of this partial process had been sufficiently established (after Fischer's experiments of 1890 at the latest), this "module" became part of the body of established knowledge on photosynthesis. The same was true of the assumption, first formulated by Erlenmeyer, that a formic acid derivative and some peroxidic compounds were involved in the process, the decomposition of which gave rise to the photosynthetic oxygen. This module constantly reappears in later model suggestions, even though the rest of the models did not resemble Erlenmeyer's original concept in other respects. Willstätter and Stoll, for example, ingeniously recombined this module with the chlorophyll-complex and the formaldehyde modules, added some causal hypotheses from their own field of expertise and thus presented a completely new amalgamation of ideas that had, in fact, been around for decades. I shall refer to this practice as the "building block strategy": scientists carefully examined their predecessors' results and then integrated into their own work whatever they found useful and acceptable.[51]

Figure I.11 (p. 56) is a schematic representation of the models discussed in this chapter and shows which modules were re-used. The arrows from X to Y denote

[50] See, e.g., Meldola (1906).

[51] The strategy resembles Lindley Darden's more broadly conceived notion of "modular subassembly", see, e.g., Darden (2002), while the term "module" in this chapter refers specifically to the established "branches" of causal graphs.

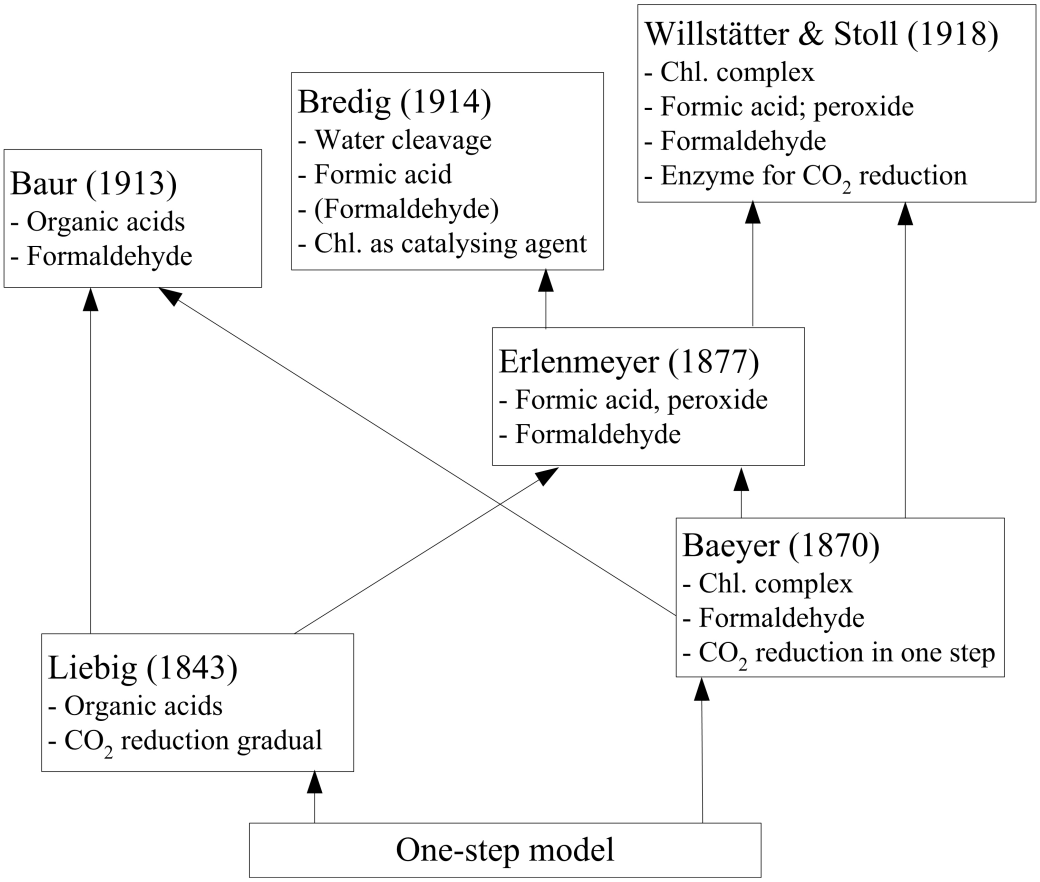

Figure I.11: The models and their "building blocks" shown in sequence.

the relationship "modules of model X were integrated into model Y" (that is, a quasi-causal relationship between different models). The one-step model is taken as the starting point for the two first major alternative models that emerged in the nineteenth century: those proposed by Liebig (1843) and Baeyer (1870). Seven years later, in 1877, came Erlenmeyer's suggestion, which was influenced, on the one hand, by Liebig's proposal of a stepwise carbon reduction via acids (although Erlenmeyer believed that formic acid was the central intermediate, something that Liebig had not even mentioned), and on the other hand by Baeyer's suggestion, as Erlenmeyer also included carbohydrate formation via formaldehyde in his model. In 1913, Baur published his proposal, which also attempted to synthesise the different approaches; this was followed in 1914 by the model advanced by Bredig, who, like Erlenmeyer, favoured the path via formic acid (and was rather sceptical about formaldehyde); finally, in 1918 the Willstätter–Stoll model was put forward, which was influenced both by Erlenmeyer's and Baeyer's suggestions particularly the latter's assumption that a complex of chlorophyll and carbon dioxide was formed.

The building block strategy, as implemented in this case, is not only a common and useful practice; it is, arguably, the only promising way to construct a complex causal graph, since no single person is capable of investigating all aspects of the

process to satisfactory extent.[52] As I shall demonstrate in my discussion of Otto Warburg in Chapter II, the building blocks, or modules, were not only taken from predecessors working in the same field; they were also taken from very different areas of science, such as photochemistry and quantum physics, which one person alone could not possibly hope to master. Using plug-ins from other disciplines was the only viable option. One can interpret this practice as a form of "cooperation" of a very impersonal nature. Scientists not only collaborate with their contemporaries, they also build upon the work of their predecessors. In fact, the history of science amply demonstrates that discoveries are not made in solitary moments of illumination but are rather the result of a long and thoughtful re-shuffling of well-known modules, combined with the successful transfer of established causal relationships to new contexts. One of the advantages of causal graphs is that they help one to elucidate the underlying processes of model dynamics, and neatly nail down what it means, in concrete examples, to stand on the shoulders of giants – namely to use the explanatory models, or at least some of the modules, of notable scientists of the past as starting points for one's own causal hypotheses.

7.4 The Principle of Plurality

Figure I.11 could give the impression that the earlier model variants were replaced by succeeding ones during the course of this chapter's time span. However, this was by no means the case. None of the models represented in the figure was dropped until well into the 1920s – not even Liebig's and Baeyer's earliest, original ideas. Scientists struggled to establish the causally relevant factors and their relationships and identify the promising parts of the different proposals, which all had their strengths and weaknesses. After 1918, the model that Willstätter and Stoll proposed was considered by many scientists to be the most promising option, and it successfully integrated a fair portion of the modules under discussion. Yet, because of the inherent impossibility of falsifying causal hypotheses, there was no way of proving that any one of the competing proposals was wrong, so none of the concurrent alternatives was abandoned: every now and then selected modules of the earlier models were revived and re-examined. I call this practice the "principle of plurality", which is a typical feature of ongoing modelling processes.

The strategy to keep as many models as possible under investigation is well-founded if one regards the model-building process as a cooperative enterprise of the whole collective. At the time, it was impossible to determine conclusively whether carbon reduction was achieved through a series of intermediates, which possibly included organic acids of one kind or another, or whether carbon dioxide was directly converted into formaldehyde or some other compound with the same oxidation state. Concurrently pursuing alternatives, as long as some uncertainty prevailed (which was usually the case), seemed a reasonable course of action. Indeed, it would have been disastrous to make too early assumptions regarding the accuracy of any one possible path. The principle of plurality was also a useful

[52] This practice was not only used to construct explanatory scientific models as dealt with in this study. Comparable practices were revealed in the construction of botanical illustrations (that is, representations of species models) around 1800, where elements from earlier images were extensively copied but at the same time modified and adapted to the new context. See Nickelsen (2000), Nickelsen (2006*b*) and Nickelsen (2006*a*).

strategy from the perspective of the individual actors. The scientists working during the period under review were struggling to establish causal relationships on the grounds of very insecure data, based on highly fallible assumptions. It was not at all improbable that an outsider module, such as the water hypothesis, might prove, in the course of time, to be the better horse to bet on. Thus, if individual players did not find it too costly, in terms of time, energy and resources, it was also worth their while to continue working on less promising alternatives – on the off-chance of striking gold!

Indeed, if the scientists working around 1900 were perfectly honest with themselves, it was not even remotely plausible that any one of their model alternatives was "accurate" in the full sense of the word. Willstätter and Stoll's model definitely had explanatory value and explained relevant sets of data with recourse to established chemical knowledge, but no experiment demonstrated the causal relationship (in fact, not even the occurrence!) of the postulated chlorophyll-carbonic acid complex; and it seemed improbable that highly poisonous compounds such as peroxides and formaldehyde could be regular intermediates of this vital life process. However, at the time it made no sense to exclude potential pathways only because some of their assumptions appeared unconvincing. Indeed, given the body of knowledge of chemistry at the time, the occurrence of the whole process of photosynthesis appeared highly unlikely, if not virtually impossible. Both water and carbon dioxide were known to be extremely stable, inert molecules, so that it was hard to believe that they decomposed at room temperature. However, since the actual existence of photosynthesis could not be disputed, there was no harm in invoking even the most improbable mechanisms to explain the process. The need to explore all model possibilities, at least conceptually, was apparently stronger than the desire to present only those causal hypotheses that were fully based on conclusive experimental reasoning.

In the end, of course, all the models discussed in this chapter were dropped (at least, by most scientists). However, this was done neither because the empirical evidence was incomplete, nor because the central assumptions appeared implausible. None of these models was ever "falsified", which was shown to be impossible anyway, if one takes these models to be descriptions of causal processes. And neither had their proponents died, to cite yet another potential reason for the success of new theories and models. Rather, in the course of time it transpired that all the models presented in this chapter could no longer be extended or modified to accommodate the new experimental discoveries being made; furthermore, new alternative models, which explained the data more convincingly, were proposed. Surprisingly, though, these models lasted for a considerable period of time. For example, the formaldehyde "module" (if one thinks solely of the causal sequence from formaldehyde to carbohydrates) was abandoned only after the first (formaldehyde-free) cyclic models had been created as a result of the radioactive tracer studies undertaken at the University of California, Berkeley (US): that is, in the late 1940s.

8 Collective versus Individually Diverging Goals

In section 6.3 it was mentioned that each of the models discussed in this chapter had a specific focus: that is, each of them was particularly detailed in some modules

of the causal graph, while other parts were treated more superficially. Thus, none of the models was intended to grasp the biochemical pathway of photosynthesis in all its complexity. As I will demonstrate in this section, these differences in focus can be explained by turning to the authors' individual interests and competencies. Remember, none of the actors discussed in this chapter was a photosynthesis researcher as such; none had even studied the process for any lengthy period of time. Rather, all of them made only one contribution to the field, which was frequently presented in one single paper, and they then moved on to other concerns: more precisely, they returned to their original, main, research goals. I will elaborate on this aspect in more detail in this section.

8.1 Photosynthesis as a Side Issue

I shall start by taking another look at Liebig. At the time that he formulated his model, Liebig was involved in the general (and rather ambitious) project to explain all agricultural processes chemically, not only in order to gain fundamental knowledge but also because he had specific applied and utilitarian purposes in mind. Among other things, Liebig was concerned with enhancing crop production and, thus, of ensuring adequate food supplies. This was the context in which Liebig started to think about photosynthesis. From this larger perspective, it is clear that he regarded the task of "explaining photosynthesis" at best as a lower-level goal or sub-goal. It was simply a step on the way to reaching his actual, superordinate goal. If one wanted to enhance crop production, for example, by developing an efficient fertiliser (which was one of Liebig's objectives) or by advising farmers how to grow their plants, it was obviously advantageous to have some knowledge about photosynthesis, the source of all plant growth.

The motivation behind Baeyer's investigations into photosynthesis was even more questionable. Around 1870, Baeyer was studying condensation reactions, including the condensation reactions of formaldehyde, and his contribution to modelling photosynthesis – namely, the formaldehyde model – was directly related to this work. This can be validated by looking at the title of the paper in which the model was published: "On dehydrogenation and its meaning for the life of plants and for fermentation processes". Chemically speaking, the "dehydrogenation" of compounds is one of the effects of those reactions that, following Baeyer's suggestion in the paper, came to be called "condensation reactions".[53] Indeed, Baeyer's famous and influential model of photosynthesis only appeared in this paper to illustrate one of the types of condensation reactions that Baeyer was discussing. Another type of condensation reaction, according to Baeyer, was central to fermentation processes. Thus, the modelling of photosynthesis was not even one of his sub-goals. Rather, Baeyer most probably realised that he could make a contribution to photosynthesis research only while he was working on the more general phenomena dealt with in the paper. I shall call this type of goal "incidental goals" (*Nebenziele*), since it is the by-product of work done while attempting to attain other superordinate goals. Typically, incidental goals are very limited and specific, and can quickly be reached on the basis of immediately available

[53] Baeyer (1870), p. 64. The original German title of the paper reads: "Über die Wasserentziehung und ihre Bedeutung für das Pflanzenleben und die Gährung".

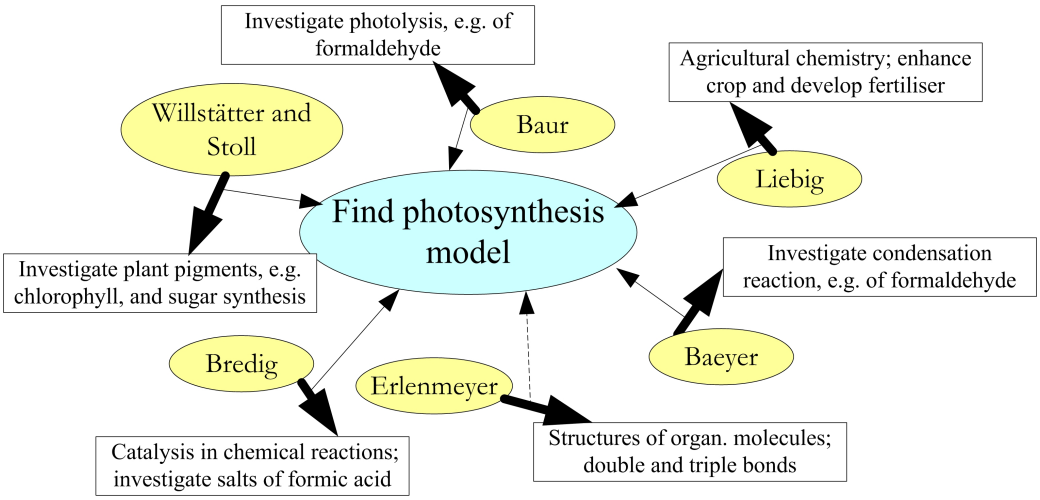

Figure I.12: The actors and their goals: diverging individual (superordinate) goals; and the sub-goal or incidental goal of finding the photosynthesis model. The thick arrows in bold indicate the relationship "X pursues the superordinate goal Y"; the thin arrows indicate that, in the course of pursuing the superordinate goal, an incidental goal or, less frequently, sub-goal (of contributing to finding the photosynthesis model) was reached.

knowledge and competencies. Having reached the incidental goals (that is, in the case of Baeyer, having published his thoughts on how the condensation reactions of formaldehyde might help to explain photosynthesis), the actors immediately go back to their original work. Baeyer's pattern of work reflects this practice: even though his photosynthesis model was the subject of protracted discussion, after 1870 he himself never again returned to it.

The other cases examined in this chapter are very similar. Erlenmeyer was, around 1877, primarily working on the structural elucidation of organic molecules and on the chemical properties of double and triple bonds. In the one paper of relevance here, photosynthesis was mentioned only in passing, as a possible application of Erlenmeyer's thoughts on "Water as an oxidising and reducing agent" (as the title of the paper reads). In 1913 on the other hand, Baur published a whole series of papers on the topic of photolysis, including, among other things, the photolysis of formaldehyde. This presumably led him to think about other problems related to photoreactions and formaldehyde, including photosynthesis. As in Baeyer's case, it did not cost Baur much in terms of resources to make a quick contribution to photosynthesis before returning to his original line of research. Bredig, meanwhile, was particularly interested in the effects of surface catalysis, and since he assumed that chlorophyll acted as a surface catalyst (like palladium or platinum), he followed this approach when framing his photosynthesis model. The latter was not an obvious topic of interest for Bredig, who was, after all, a physical chemist. The findings he presented provide some indication as to how he chanced upon the subject: while working on formates (the salts of formic acid), Bredig had

found that, under the influence of a catalysing agent, they could be oxidised to the salts of carbonic acid. Thus, an obvious conjecture to make from this was that this reaction might also work the other way round, that is, reduce carbonates to formates, and that this could then be assumed to be one of the processes involved in photosynthesis.

Finally, Willstätter and Stoll had already been working on the chemistry of plant pigments, in particular chlorophyll (they had, for example, established that magnesium was an integral part of chlorophyll and they were the first to find a practicable method for isolating chlorophyll from plants), for more than ten years when they published their model.[54] It is not too surprising then that this work prompted them to make contributions to the mechanism of photosynthesis – and neither does it come as a surprise that the focus of their model was the role of chlorophyll. Yet, even then, "finding the photosynthesis model" was evidently not their first and primary goal, as is made clear in the introduction to Willstätter & Stoll (1918):

> Even though our experiments may contribute to describing more precisely the processes of [carbon dioxide] assimilation, at the same time they clearly give a negative answer to the question as to whether it is already possible to realise this assimilation outside the living cell. It is too early for experiments of artificial assimilation under the influence of chlorophyll. This is not really a negative conclusion, it is a positive finding that ought to inspire and point the way to new work.[55]

This rather defensive-sounding paragraph (in which the authors appear to be trying hard to raise their spirits in view of their failure to achieve their goal) reveals that Willstätter and Stoll's primary goal was to realise artificial photosynthesis: to find a way of reducing carbon dioxide to carbohydrates in a light-driven reaction under the influence of chlorophyll, but without the rest of the plant cell. And it was only in order to reach this goal that Willstätter and Stoll found it necessary first to clarify how the process operated in plants. "Finding the (natural) photosynthesis mechanism" was for them, too, a sub-goal in their quest to achieve artificial photosynthesis.

8.2 Constructive Research Opportunism

Thus, it seems that all the work carried out on photosynthesis in the nineteenth century and presented in this chapter can be interpreted as a by-product (or spin-off) of the scientists' work on other projects. At some point in their research, Baeyer and Erlenmeyer among others must have realised that, based on what they had achieved so far (in other topics), they could easily make a contribution to photosynthesis research. Following a suggestion by Gerd Graßhoff (in a personal communication), I refer to this behavioural pattern in this study as the principle of "research opportunism". The following maxim formulates the principle's underlying rationale: "Contribute to solving an interesting – and important – research problem,

[54] See their first monograph: Willstätter & Stoll (1913).
[55] Willstätter & Stoll (1918), preface, p. IIIf.

only if you can do so without being distracted for too long from the pursuit of your principal goals."[56]

It seems that many scientists are quite willing to make a contribution to an open question if the opportunity arises, even though they might never develop more than a passing interest in the pertinent subject. They might provide explanatory approaches, introduce new methods or apply concepts from their actual area of research; but immediately afterwards, they will return to their own field.[57] At first glance, Willstätter and Stoll seem to have been exceptions to this rule, since they spent so many years working on questions related to photosynthesis. However, if one looks at their case a little more closely, it is clear that most of the time they studied only the structure of chlorophyll and its behaviour in different circumstances, and that it was only in the final chapter of their second book that Willstätter and Stoll turned to the mechanism of photosynthesis – which they only examined in order to find out how to synthesise sugars in the test tube (see above). This also explains why Baeyer never turned to photosynthesis again, even though he left many problems unsolved; and the same is true of Liebig, Erlenmeyer, Baur, Bredig, Hofmann and Schumpelt.[58] It was not that these men deliberately wanted to hold things back; rather, they had published all they had to say on photosynthesis. Given the limited resources in terms of research time, infrastructure and money, pursuing an incidental goal is only worthwhile from the actor's perspective if it can be reached quickly and with a minimum of additional effort. This explains why all the contributions discussed in this chapter closely corresponded to the actors' individual, superordinate goals and why the models they proposed had very different foci.

Photosynthesis research around 1900 is, therefore, a prime example of the principle of research opportunism. It is easy to see why this pattern of behaviour was recommendable. Although many people were interested in photosynthesis and contributed some findings to explaining the process, hardly anybody at the time made the subject their centre of interest. As was mentioned earlier, the whole field of metabolism studies was highly problematic at the time, since there were no methods through which one could gather direct information on the course of the pathways. Data were scarce and the interpretations thereof disputed. It would have been highly unreasonable to make a theme in which success was so uncertain the sole focus of one's research. Contributing to the subject in an "opportunistic"

[56] The principle can be marvellously illustrated by an episode remembered to have happened by one of the giants of photosynthesis, William Arnold, in 1950: "In June [1950], Dr Bernard Strehler came to the [Oak Ridge National] Laboratory [in Tennessee, US]. Strehler had a brand-new Ph.D., a tremendous amount of energy, and lots of ideas about almost everything. One day he appeared in the laboratory door and said, 'Arnold, how would you like to make one of the fundamental discoveries in plant physiology?' My answer was: 'OK, if it won't take too long'." See Arnold (1991), p. 79. The collaboration eventually led to the discovery that adenosine triphosphate ATP is synthesised in the chloroplast. For details, see Chapter VI.

[57] See Graßhoff (1998*b*) for a case study from astrophysics in which he discusses this phenomenon more generally.

[58] Prompted by findings that Stoll made, Willstätter and Stoll both contributed once again to the field around 1932; see Stoll (1932) and Willstätter (1933). Other scientists who, at the beginning of the twentieth century, made a single contribution to photosynthesis research from their own field of expertise, include Emil Fischer (see earlier in the chapter), Felix Hoppe-Seyler, Walther Nernst, Walter Noddack and Fritz Weigert.

manner was the best option, both for the individual scientist, whose costs were limited while the potential gains were high, and for the collective as a whole, since this was, after all, a viable option for keeping the topic alive until more adequate methods became available.

This moment came with the rise of manometry in photosynthesis research after 1920. Yet, even though it became principally possible – and reasonable – to concentrate one's efforts on photosynthesis, which by this time was no longer considered a high-risk field of study with almost no hope of success, the subject remained a side issue in plant physiology (and other disciplines). The set of people that spent their professional lives on photosynthesis studies remained limited: only a small number of research groups made photosynthesis the central focus of their work, although they were extremely successful. In addition to the specialists, there always remained a number of "opportunistic" contributors, who were only marginally interested in photosynthesis, yet did not hesitate to take a shot at it if their area of expertise made it appear worthwhile. It will transpire in the following chapters of this book that both parties played an important role in solving the problem of photosynthesis.

9 Summary

The focus of this chapter was the early attempts made to model the photosynthetic mechanism in the form of a biochemical pathway. First, the general approach of this study was introduced: to analyse and reconstruct explanatory models in the form of causal graphs, and to interpret their construction as the result of the cooperative work that took place within a research collective. It was demonstrated how different hierarchies of research goals resulted in the formation of different hierarchies of research collectives, which were determined by the actors' competencies and interests. This informal, problem-driven organisation of a "scientific community" is taken to be typical of many areas of science, and it is, to some extent, independent of more formal types of organisation, such as research teams and institutes. Individual players (or, later, research teams) typically specialised in a certain problem within a larger project and sometimes even in a certain approach to the problem. In other words, they set themselves a sub-goal that might contribute to achieving a superordinate goal. However, if the opportunity arose, the group (or individual members of the group) was ready to contribute to solving other problems on the way: this was introduced as the strategy of "research opportunism". If one is working on the reaction of formates, one might as well find out, as a side issue, whether formates can be produced from carbonates, which might be relevant to photosynthesis. The actors in this chapter immediately returned to their original research goal, although in later chapters of this book we shall come across several research opportunists who found themselves unable to break free from their incidental goal. Pursuing a goal indirectly could, at times, result in an unforeseeable change in research priorities.

The discussion of the examples in the chapter also revealed how the models, which were published in a temporal sequence, were interrelated. Explanatory models, such as the photosynthesis models, are built upon a body of common knowledge, however limited this common knowledge might be, which, in the form of

a model prototype, is taken to be the starting point and then extended and modified in one way or another. The first suggestions for extending a model typically result from the transfer of causal knowledge from one field to another. The underlying assumption is that the causal processes are of the same type: this justifies at least a tentative transfer of causal knowledge from one context to the other. This transfer typically concerned individual modules, that is, branches or intermediate sections of the causal graph that cover some of the processes involved in photosynthesis. In the course of time, these modules were usually integrated in an unaltered shape into the new model, modified or completely changed. This process, which I have called the "building block strategy" in this study, was common practice in the decades immediately after Baeyer first made his proposal: several models were developed, all of which comprised a different set of the available modules (or building blocks), combined with one or two new ideas.

A great variety of these variants was pursued for a surprisingly long period of time, even though some of the models did not initially appear at all promising. No model seemed too contrived to explain the extraordinarily strange reaction of the conversion of two of the most stable molecules – carbon dioxide and water – into organic substances at room temperature. And since there was considerable uncertainty about the causal relationships involved, it only seemed advantageous to abandon potential pathways if it was absolutely clear that they could no longer be sensibly extended and adapted, in order to accommodate the advances in empirical and theoretical knowledge. This is named here the "principle of plurality", which plays out its strength particularly in those situations where scientists are working on extremely shaky ground and are unable to carry out difference tests from which causal relationships might be deduced. It seems then that, in this period of total uncertainty, the only criteria the model hypotheses had to meet were that: first, they complied with accepted theory, including the earlier established causal knowledge of the process; second, they successfully demonstrated the assumed relationships in some context (such as the formation of formaldehyde from carbon dioxide in the test tube); and, third, they had the capacity to explain causally a fair share of the relevant empirical data. It would have been inappropriate to demand that positive evidence be gathered from the system under investigation for every single causal link; scientists would then have been compelled to stop working on the problem.

Which of the models or individual modules survived the longest depended largely on their capacity to accommodate the new evidence and new theoretical knowledge that arose during the course of the decades. The formaldehyde module, for example, did very well in this respect. It was adapted, extended and modified without losing its explanatory power. However, in the face of growing evidence on the energy requirements of the process, which will be outlined in the following chapters, the model came up against insurmountable problems. The interest of most photosynthesis researchers had, by then, shifted anyway, since promising new approaches had emerged to address the process from a completely different angle, namely in terms of its reaction kinetics, measured with the help of manometry. Given this situation, the cumbersome and largely speculative search for potential carbonic intermediates lost its former attraction. But the formaldehyde model

was never actually falsified. Although the failure to detect formaldehyde and demonstrate its causal relevance in plant metabolism was a serious problem, it was not fatal. Negative results of this kind cannot disqualify causal hypotheses. They only mean that the difference test situation has so far not produced a difference in outcome. And the only conclusion that one can draw from a result like this is that the result is inconclusive. There was always the possibility that not all the required conditions had been realised in the test situation; and there was also the possibility that there existed an alternative path of photosynthesis through formaldehyde. The only option in situations like these was to develop a powerful explanatory model without formaldehyde that was so much better suited to accommodating all further findings that the older alternatives consequentially fell into oblivion.

In view of this situation, it initially seems that the problems and solutions of all the actors in this chapter eventually faded away without trace, and that they had no influence on today's "accurate" photosynthesis model. They were, so it appears, simply more examples to be added to the many impasses in the history of science, which perhaps explains why these models have never been treated *in extenso* in any of the standard works on the history of biochemistry. Nothing could be more off the mark. To be sure, the story is not a continuous one. The optimistic endeavour of finding the intermediates of the process of photosynthesis by way of the crude methods available was dropped in the 1920s, and only taken up again when radioactive tracer molecules became available in the late 1940s. However, the results that were obtained continued to be of importance for future work in the field. Not only did researchers believe that formaldehyde was among the key intermediates; the causal knowledge that was gained in these years – concerning reaction steps and potential mechanisms, the types of interactions between certain molecules, and so on – was retained and remained available for later generations. It became part of the body of "generally established knowledge" upon which later researchers would draw in their work on plant metabolism and other quite different subjects, despite the fact that the models discussed in this chapter were, on the whole, wrong.

Chapter II

OTTO WARBURG AND THE TURN OF MANOMETRY (1912–25)

In this chapter the focus shifts from the many attempts to explain the process of photosynthesis to a close-up of a particular individual, namely the German physiologist Otto Warburg. His contributions to the field marked a turning point in twentieth-century photosynthesis research: he introduced a number of revolutionising new techniques to measure the rate of photosynthesis (which resulted in the move to kinetic studies of the process), he put forward a new model of the mechanism, and he added a completely new perspective to the subject by attempting to establish the efficiency of the process in terms of the quantum requirements of photosynthesis.

The example of Warburg finely illustrates the far-reaching consequences that opportunistic behaviour can have on research. The application of one experimental technique – measuring metabolic processes using manometric methods – dominated virtually his entire career and, beginning with his seminal work on cell respiration, he was constantly searching for the effect of metal-containing enzymes acting on internal cell surfaces. With this admittedly limited range of concepts and techniques, Warburg was able to contribute, at the highest level and with great success, to fields as diverse as cell respiration, photosynthesis and cancer research. In this chapter I shall trace the nested hierarchy of goals that brought Warburg to make his contributions to the field of photosynthesis research. I will also analyse how Warburg came to work in the field of photosynthesis, which sources he used to compose his model hypothesis and why he decided to measure the quantum efficiency of the process. The general objective of this analysis is to gain a better understanding of the reasons why individual actors pick out certain sub-goals within a field of study and how their specific background shapes the contributions they make.

To this end, three different sources of inspiration are explored: Warburg's early research into cell respiration; his father's work on the quantum yield of photochemical reactions in general; and the way in which Warburg reacted to the photosynthesis work carried out by Richard Willstätter and Arthur Stoll (which was summarised in Chapter I). How Warburg ingeniously availed himself of fragments taken from these contexts, recombined them in a new and innovative way and explained his experimental findings is a marvellous example of the successful use of the building block strategy.[1] I shall begin by outlining Warburg's career up until 1920, focusing in particular on those details that are of most significance in my reconstruction of the story. An examination of Warburg's personal background will help us to understand how he chose the building blocks that would determine his contributions to the field.

[1] See also Nickelsen (2009) on this point.

1 Otto Warburg (1883-1970)

Otto Warburg was one of the most successful and influential biochemists of the twentieth century.[2] Born into a middle-class German family of partly Jewish origin, his father was the experimental physicist Emil Warburg, one of the most eminent scientists of his time. In 1905, after having held various academic positions (in Strasbourg, Freiburg im Breisgau and Berlin), Emil Warburg was appointed President of the Physikalisch-Technische-Reichsanstalt (the Physical and Technical Institute of the Reich), or PTR for short, in Berlin, where he remained for the rest of his working life, that is, until 1922. This Berlin-based appointment would also have a momentous influence on the career of his first child and only son.

Otto Warburg had a typical middle-class German education, attending a humanistic *Gymnasium*, before going on to study chemistry at Freiburg im Breisgau in 1901. After having spent a few terms there, Warburg moved to Berlin, where he continued his studies in the laboratories of the organic chemist Emil Fischer (the same Fischer who was mentioned in Chapter I: a former doctoral student of Adolf von Baeyer; in 1890 he synthesised sugar via formaldehyde). While working in Fischer's laboratory, Warburg earned his doctoral degree in chemistry in 1906.[3] During the years 1905 to 1906, Warburg received additional training in his father's laboratory at the PTR, where he became familiar with, among other things, the vacuum bolometer, an apparatus devised by Emil Warburg to measure light intensities.[4] This was one of the measuring instruments that Otto Warburg would later use in his research work on the quantum yield of photosynthesis.[5]

Warburg could then have embarked upon a career as a chemist; however, not satisfied with only a knowledge of pure chemistry, he chose to broaden his education by studying medicine at Heidelberg, with, among others, the well-known physician and physiologist Ludolf von Krehl. He earned a second, medical doctorate in 1911, and in 1912 attained his habilitation. Warburg stayed with Krehl for one more year , and it was at Heidelberg that he began his successful research work on the processes of cell oxidation. Warburg's findings in this field were to bring him his first major breakthrough as a scientist in his own right. And they were to have lasting consequences: Warburg was awarded the Nobel Prize for Medicine or

[2] For general accounts of Otto Warburg's life and work, see, e.g., Krebs (1979), Henning (1987), Höxtermann & Sucker (1989), Werner (1991) and Höxtermann (2001); selected parts of his sister's personal notes were published in Rüskamp (1989). Warburg's contribution to the theory of cell respiration, as reflected in his correspondence with, e.g., the physiologists Jacques Loeb, Leonor Michaelis and Otto Meyerhof, is treated in Werner (1996), while Kohler (1973*a*) investigates the background of Warburg's concept of *Atmungsferment*. On Warburg's experimental methods in photosynthesis, see also Hoppe (1997), p. 19f.

[3] It has not escaped the notice of Warburg's biographers that, in his botany examination, Warburg demonstrated only "satisfactory" knowledge of "carbon assimilation", unlike the excellent results he received in all his other subjects. Clearly, the young Warburg had not yet developed a passion for what would later become one of his main research themes! See Höxtermann & Sucker (1989), p. 21, for a facsimile of the exam's documentation; a transcription can be found in Werner (1991), p. 24. The original document is preserved in the Archives of the Humboldt University of Berlin, shelf mark Phil. Fak. No. 411, folio 210.

[4] See, e.g., Warburg, Leithäuser & Johansen (1907) and Warburg (1909).

[5] For an autobiographical account of this period, see the taped interview with Warburg of 1966, quoted in Krebs (1979), p. 94f.

Physiology in 1931, largely because of the studies in cell oxidation that he resumed in the 1920s.

In 1913, at the age of thirty, Warburg returned to Berlin, having being appointed head of his own research department (his first such appointment) in the newly founded Kaiser Wilhelm Institute (KWI) for Biology in Dahlem.[6] However, since the institute's new building was not completed in time, Warburg had a gap to fill between leaving Heidelberg and starting at the KWI. He busied himself, first by working again in the PTR's radiation laboratory, where he undertook some photochemical work, and then in the physico-chemical laboratory of Walther Nernst at Berlin's Friedrich Wilhelm University, where Warburg apparently worked on the oxidation potentials of living cells.[7] Both episodes would lay the groundwork for his later research into photosynthesis.

Warburg's first years in Berlin were interrupted by the outbreak of the First World War in 1914. He immediately volunteered and started serving in the Prussian Horse Guards, who were involved in activities near Germany's Eastern Front. Warburg remained in service until 1918, yet he returned to Berlin before the official end of the war – presumably due, at least in part, to a letter he had received from Albert Einstein, urging him to return home.[8] Warburg apparently agreed to this suggestion , and so his father and the plant physiologist Carl Correns, Warburg's superior at the KWI for Biology, entered upon a lengthy correspondence with the Ministry of the Interior, requesting Otto Warburg's release. In addition to citing general scientific reasons, both Emil Warburg and Correns stressed that Otto Warburg's release would benefit the public, since, they argued, the resumption of his research work would very likely yield results that would help improve the population's nutrition (most probably alluding to Warburg's work on photosynthesis, with which both his father and Correns were already familiar).[9] Eventually, the request was successful and in October 1918 Warburg resumed his research in the by-now-completed laboratories of the KWI. Among the first papers that Otto Warburg published after having returned to Berlin were his articles on photosynthesis, which was a vigorously debated theme at the time.

Otto Warburg embarked upon the field of photosynthesis research with two closely related articles, published as Warburg (1919) and Warburg (1920*b*), in which he dealt with the general mechanism of the process. In Warburg (1921) he supplemented these papers with a comprehensive synopsis of his findings up to this

[6] On the early history of the society, see, among many other publications, Vierhaus & Brocke (1990); on the KWI for Biology, see also Sucker (2002).

[7] See, e.g., Werner (1991), pp. 75 and 113.

[8] Albert Einstein wrote a letter to Warburg, on the initiative of the latter's mother, in which he tried to convince Warburg that he was more urgently needed in Berlin than at the Front; Warburg, it seems, was won over. This letter can be found in Schulmann, Kox, Janssen & Illy (1998), pp. 694–697 (Nos. 489 and 491). The letter is also transcribed in Krebs's biography of Warburg, which also shows part of it in facsimile; see Krebs (1979), pp. 20–23.

[9] See Werner (1991), pp. 121–122. As the science historian Petra Werner explains, during this period German politicians were considering unconventional sources of nutrition in an attempt to counteract the nation's growing problem of undernourishment; one suggestion was to follow the Japanese example of exploiting marine algae, similar to those that Otto Warburg used in his photosynthesis experiments. Other suggestions of the committee, which was led by Emil Fischer and had been set up to deal with the problem, included using reed or couch grass.

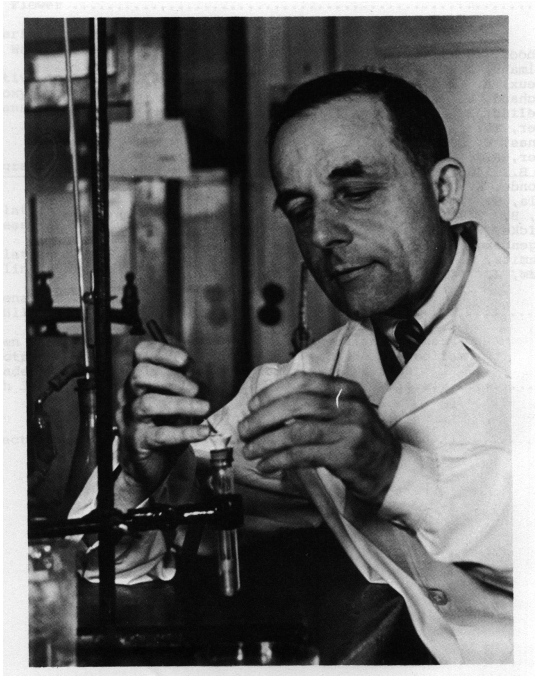

Figure II.1: Photograph of Otto Warburg (1883-1970), taken around 1920.

point. Only a year later was the first account of the quantum yield of the process, that is, of the efficiency of photosynthesis, published as Warburg & Negelein (1922). This was followed by Warburg & Negelein (1923), a second paper on the influence of the wavelength of light on photosynthetic efficiency. Both these papers were co-authored with Warburg's long-standing collaborator Erwin Negelein. Warburg (1925), in which Warburg revised some of his earlier conclusions, is another synopsis of great value.

The advances Warburg made in these papers were enormous. He revolutionised the field through introducing a number of new techniques that were quickly to become standard practice in photosynthesis research and remain so until the 1970s. These included the use of manometric rather than gasometric or titrimetric methods for measuring the rate and progress of photosynthesis. To fully exploit the advantages of this new technique, Warburg also replaced the use of leaves and whole plants as test organisms with the unicellular green algae *Chlorella*, which to this day is a well-known model organism in photosynthesis research.[10] The prompt inclusion of Warburg's experimental protocols in the textbooks and manuals of the period is evidence of how quickly his methods became standard practice in the field.

In addition, Warburg also employed sophisticated photophysical techniques, such as bolometry, absorption methods and intermittent illumination by means of rotating sectors, which required not only special skills but also specific instrumentation. He was also the first person to use inhibitors systematically in order to

[10]See Zallen (1993*a*) for a thoughtful discussion about the use of *Chlorella* algae (and others) as test organisms in photosynthesis research.

discover more about the biochemical process of photosynthesis. From the results of his research, Warburg proposed a mechanism that involved the formation of a "photolyte", a concept that he adopted from contemporary physics, denoting substances that are decomposed by photolysis – indeed, it was his father Emil who had introduced this concept to science in 1917.[11] And Warburg also brought a new perspective to debates of the period by determining the energetic efficiency and, as a consequence, the quantum requirements of photosynthesis – one of the few parameters at the time that strictly limited the range of possible model alternatives.

2 New Materials and Methods

The development of appropriate experimental techniques and apparatus played a central role in Warburg's career. Warburg was keenly aware of the need to make precise measurements in metabolic studies; and he was one of the first to accomplish this in a satisfactory manner. For his work on photosynthesis, Warburg made particular use of bolometry, absorption measurements, manometry and the adequate growing of *Chlorella* algae, none of which was easy to master. I shall now give an outline of the most important technological innovations that Warburg introduced to the field of photosynthesis research.

Manometry

Before Warburg introduced manometric methods to photosynthesis research, techniques were employed to determine gas exchanges with sensitivities measured in the range of millilitres of gas. This meant that, in order to be able to measure the minimum oxygen level in experiments, one had to use large samples or even whole plants. Thus, large areas of material had to be illuminated for a relatively long period of time. Warburg's method, by contrast, offered an enormously increased sensitivity, capable of measuring *micro*litres of gas exchange.[12] Consequently, one could not only greatly reduce the sample size and the duration of experiments but one could also utilise smaller and more manageable light beams, all of which led to a far better control of the whole experimental set-up. Warburg's main incentive for designing new techniques and instruments was to satisfy the homogeneity condition (although he would not have used this term); and he firmly believed in his ability to achieve this goal, given the appropriate instrumentation and a well-designed experimental set-up. The following anecdote can be seen to be characteristic of this approach. In 1928, Warburg gave a seminal talk on the spectrophotometric analysis of the heme molecule. In the discussion, the chemist (and Nobel laureate) Richard Willstätter harshly criticised the application of spectrophotometry to such complex structures as cells, whereupon Warburg replied: "If one finds appropriate reactions specific for the cell component which one wants to analyse, the rest of the cell is part of the test tube."[13]

The core instrument of Warburg's manometric technique was a modified version of the Haldane–Barcroft blood gas manometer (first described in 1902) in which

[11] See Warburg (1917).
[12] See Myers (1974), p. 420.
[13] Quoted in Nachmansohn (1972), p. 5.

Warburg analysed either very thin slices of living tissue (for his studies in respiration) or cell suspensions (for his research into photosynthesis). Although Warburg had started his manometric studies with a basic version of this instrument, which his father Emil had devised in 1900 to measure the velocity of ozonisation processes,[14] he had become familiar with a more sophisticated version of the instrument in March 1912 during a brief spell at the Cambridge laboratories of Joseph Barcroft.[15] The Haldane-Barcroft instrument brought definitive advantages to the investigation of physiological processes – among others, the fact that the gas volume was kept at a constant value throughout the measuring process so that the arising pressure could be used to infer the amount of newly produced gases. Another special feature were the valves that allowed one to connect the manometric vessels at any time with the surrounding atmosphere.[16] Figure II.2 (p. 73) is an illustration of one of Warburg's own manometers, together with the specific vessel or glass trough that had to be used with it, while figure II.3 (p. 73) is a sketch of the complete measuring device.

The principal procedure was quite straightforward: a suspension of unicellular algae, which had been grown under controlled conditions, was poured into the flasks, which were then connected to a manometer. This combination of manometer and flasks was then mounted on a water bath (the flasks were mounted on the interior of the bath, the manometer on the exterior) in order to keep the temperature of the suspension at a constant value. In this position the vessels were shaken to achieve homogenous conditions for all the cells at any time. Illuminating these vessels (with measured light intensities) initiated the different processes of photosynthesis, which gave rise to the evolution of molecular oxygen. The effect – namely an increase in pressure in the system – was measured by noting the changes in the height of the capillary fluid in the adherent manometer. A set of rather simple equations could then be employed to calculate the amount of oxygen produced, taking into account the relative change in manometer fluid, the relationship between the molecules of oxygen evolved and the pressure produced as well as other constants related to the vessel. Almost all of Warburg's path-breaking studies were carried out using this technique, which he continued to develop throughout his career. Its simplicity and its broad range of applications soon made it part of the standard apparatus of every physiological laboratory.

Chlorella

The use of manometric techniques led Warburg to reconsider the test organisms suitable for the study of photosynthesis. There were some major disadvantages to using the leaf tissue of higher plants, in addition to the difficulty of ensuring homogenous illumination (see above). First of all, heavy diffusion was a problem. As leaf tissue slices always comprise several cell layers, the oxygen produced in the

[14] See Warburg (1900).

[15] Sir Joseph Barcroft (1872-1947), Professor of Physiology at Cambridge; elected Fellow of the Royal Society in 1910.

[16] A comprehensive account of his early manometric techniques can be found in Warburg's book on tumour metabolism, published in 1926. See Warburg (1926), p. 1ff. Kok (1960) provides an overview of how the techniques Warburg used in his photosynthesis studies developed over time. For a brief and very accessible introduction, see also, e.g., Allen (1975), p. 173f.

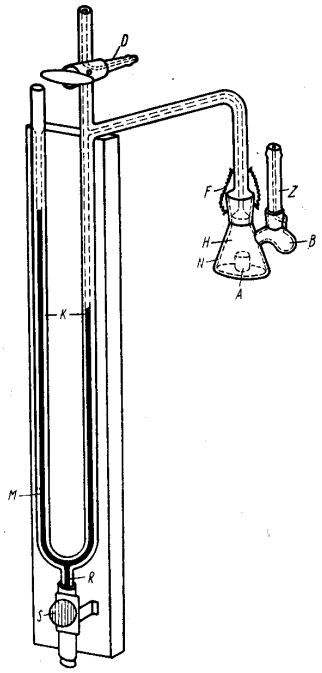

Figure II.2: An illustration of a manometer used by Otto Warburg in his photosynthesis experiments. Reproduced from Warburg (1926), p. 1.

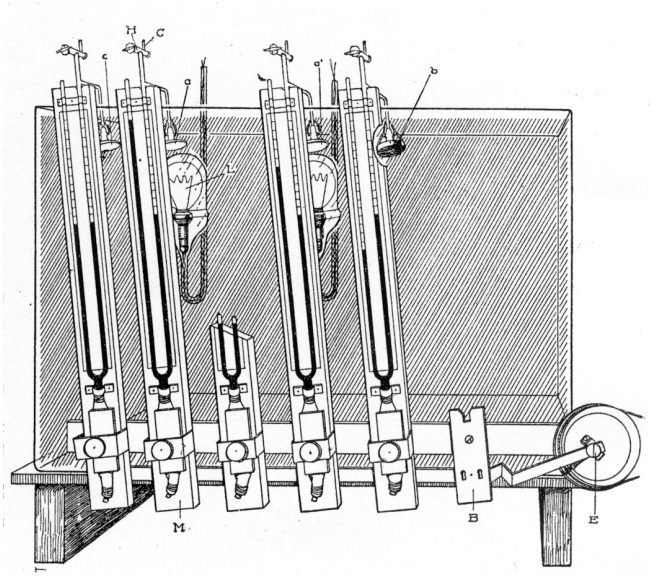

Figure II.3: A drawing of the complete measuring apparatus (which became known as "Warburg Apparatus"). The manometers are mounted on a thermostat, so that the vessels can be illuminated with light bulbs from below. A v-belt connected to an electric motor, part of which can be seen on the right of the illustration, oscillates the manometers. Reproduced from Warburg (1919), p. 245.

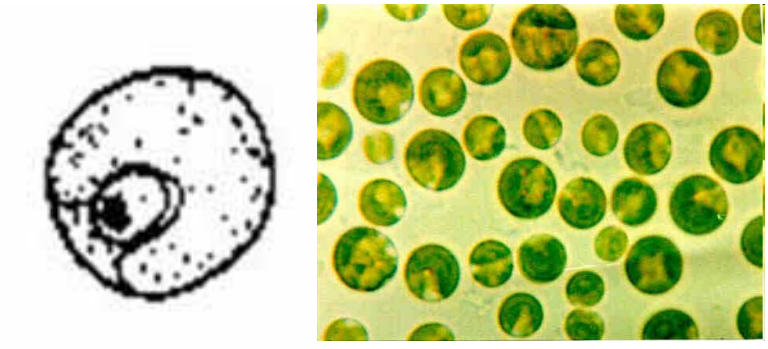

Figure II.4: A sketch of *Chlorella* sp. (left) and a microscopic photograph of a suspension of *Chlorella* cells (right).

interior cells had to diffuse through a great many other cells before it entered the suspension. This resulted in significant inaccuracies in the measurement of the gas exchanges. Second, the parameters of the micro-environment of the interior cells could not be controlled: neither temperature nor carbon dioxide pressure inside the tissue could be taken to be the same as in the rest of the vessel. In his search for alternatives, Warburg finally decided to use a unicellular freshwater green alga as his test organism.[17] As Warburg put it: "After some preliminary trials I kept to a round immobile green alga of 3 to 5 micrometers in diameter, which multiplies by successive fission without developing clusters or movable cells, similar to an alga described as '*Chlorella*' in the literature."[18]

Chlorella (see fig. II.4) had many practical benefits. It is relatively easy to grow in large quantities and the alga's chloroplast occupies half the cell volume, which means that a large proportion of the plant material used is photosynthetically active and the yields relatively high.[19] The main advantage, however, was of a methodological nature. At the time, unicellular algae were the smallest organism known that were capable of carrying out the full photosynthesis process (it would

[17]Warburg (1919), p. 231. It is highly probable that Otto Warburg was helped in his choice of organism by the mycologist Ernst Georg Pringsheim, who was one of the leading algae experts of the time. In an autobiographical account, Pringsheim reports how he was approached by Emil Warburg to participate in the photosynthesis experiments being carried out at the PTR. Pringsheim declined the offer but he was still the obvious person to turn to in search for an appropriate single-cell model organism. Pringsheim (1970).

[18]ibid., Warburg (1919), p. 231. Quoted also, originally in German, in Werner (1991), p. 148.

[19]Cf. Manning (1938), p. 120, FN 3.

not become possible to isolate chloroplasts, which would later become the preferred living structure for testing, until the 1920s; and, anyway, most scientists doubted whether the whole array of photosynthetic reactions could be carried out in the chloroplasts).[20] The small size of the algae meant that the paths between the reaction sites in the cell and its environment were short, so that Warburg's observations were no longer affected by diffusion time lags: turning the light on immediately produced oxygen, while turning the light off immediately stopped oxygen production. This was a pre-condition for the use of flashing light experiments, in which Warburg studied the effects of light and darkness given at short intervals. The temperature of the cell interior was practically identical to the temperature of the suspension and, finally, the experiments could be carried out using comparatively small quantities of light: in an algae suspension, light readily penetrates the cells without being absorbed by non-photosynthesising regions or reflected away by a leaf's surface. Thus, the main experimental parameters, such as temperature, the gaseous and liquid environments, and light intensity, could all be controlled and rapidly and homogeneously changed. This implied that, in almost all respects, using *Chlorella* as a test organism enormously increased Warburg's control of the homogeneity condition and hence made his conclusions more reliable. The main advantage was not that the measurements were more "accurate"; rather, confounding factors were minimised thanks to a much more comprehensively controlled set-up.

In the years that followed, *Chlorella* algae became a popular test organism for photosynthesis studies in other laboratories. According to Zallen (1993*b*) two main factors accounted for this. First, Warburg was one of Germany's leading scientists. Visitors from all over the world came to his laboratory, and then took to using *Chlorella* on their return home. In addition, some of the period's most influential photosynthesis researchers were trained in Warburg's institute, including the Americans Robert Emerson and Charles Stacy French, who spread the technique across the United States. Second, most of the crucial experiments, in particular those experiments undertaken during the course of the controversy on the maximum quantum yield of photosynthesis (which is the subject of Chapter IV of this book), were carried out on *Chlorella* cells, so that a lot of knowledge on the behaviour of this alga accumulated over the years.[21] This point deserves a little more reflection. In the course of time it was found that the physiological state of the algae and their growth history strongly influenced their photosynthetic performance. This meant that it was extremely important always to grow the same strand of algae under the same (favourable) standard circumstances if one wanted to comply to the homogeneity condition. Once appropriate growing conditions had been defined for *Chlorella* – a task which turned out to be far from easy – many scientists were reluctant to change the test object again. Any modifications made, either to the growing conditions or to the organism itself, would have required a lengthy investigation into the comparability of the new situation with the established experimental standard.[22] Because of the large amount of information

[20] See Zallen (1993*b*), pp. 271–273.

[21] See Zallen (1993*b*), p. 273.

[22] See Creager, Lunbeck & Wise (2007) for a recent volume on the "model systems approach" as the editors call it: the practice of using organisms that have become standard test objects or

collected about its organismic properties, *Chlorella* has remained an important test organism to this day, even though isolating chloroplasts no longer poses a problem.[23]

Buffer solution

Using unicellular algae for manometric experiments required finding a more sophisticated solution to the carbon dioxide problem (that is, to keeping the carbon dioxide concentration at a constant value), since the usual carbonate-bicarbonate buffers were, because of their (slight) alkalinity, potentially harmful to the algae. Therefore, Warburg developed a specific buffer with an almost neutral pH and an extremely low carbon dioxide concentration, consisting of 15 parts one mole solution of Na_2CO_3 and 85 parts one mole solution of $NaHCO_3$.[24] (Note that it was only in the course of his quantum yield studies that Warburg started to use acidic, phosphate-containing buffers.)

Bolometry

Warburg also employed sophisticated bolometry in his photosynthesis studies. He had learned about this technique while working in his father's laboratory, although at the time most biologists, and even chemists, were not familiar with it. A bolometer is a relatively sensitive device for detecting and measuring radiation intensities. The instruments in use around the year 1900 consisted of a Wheatstone bridge, the two branches of which were connected to very thin (0.0025 mm) strips of metal (steel, platinum, palladium, and so on), a battery and a galvanometer for measuring electrical currents. If one of the two metal strips was exposed to radiation, the metal heated up, which increased its electrical resistance. Consequently, the galvanometer would detect a certain voltage between the two parts of the system, proportional to the amount of radiation energy incident on the metal. By 1907, bolometers could detect temperature changes of 0.00001° Celsius.[25] It was Emil Warburg who improved the device when he invented the vacuum bolometer, which was first described in 1907.[26]

Rotating Sectors: The Flashing Light Technique

Warburg also adapted techniques of intermittent illumination, which allowed him to study photosynthesis under conditions in which the light reactions limited the velocity of the process. To this end, Warburg used "rotating sectors": a disc with one or more sections was placed between the light source and the algae, so that part

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a reference to case studies that have acquired exemplary status within a discipline. The editors argue that model systems are used as "models for" something if the system under investigation is too complex to construe a "model of" it. The aspect discussed here, how the choice of standard organisms enhances the reliability of causal inferences, complements the analyses given in the volume.

[23] This does not, of course, mean that all the scientists working in photosynthesis research exclusively used *Chlorella*. Other algae that were used as test organisms included the species of *Euglena*, *Scenedesmus* and *Porphyridium*, while in the field of genetic engineering *Chlamydomonas reinhardtii* has, more recently, become the standard model organism; see Zallen (1993*b*), p. 275ff.

[24] See Werner (1991), p. 148.

[25] See, e.g., the contemporary entry on "bolometer" in *Meyers Grosses Konversationslexikon*, 6th edition, Vol. 3, 1907, p. 184.

[26] See Warburg et al. (1907).

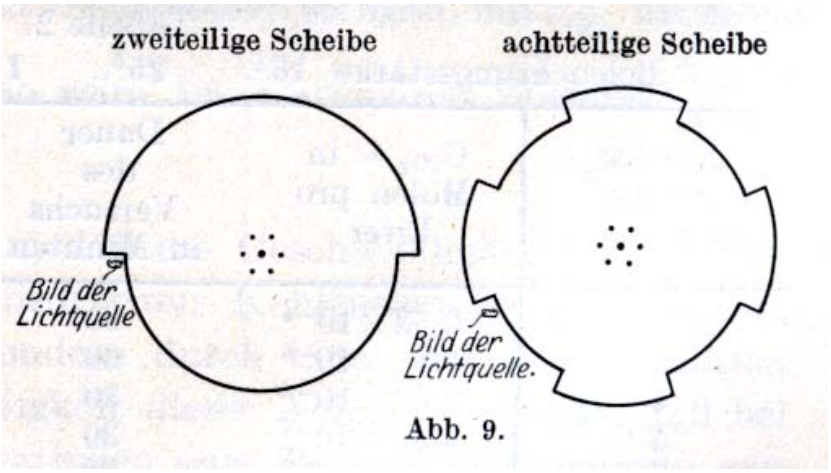

Figure II.5: A drawing of Warburg's rotating sectors in Warburg (1919), p. 251, fig. 9. Two different variants are depicted: on the left, a disc divided into two halves (i.e., two periods: one dark, one light) is shown, while the disc on the right is divided into eight sections (resulting in four dark and four light periods each). The tiny rectangle to the left of each disc marks the position of the light source.

of the light could be screened off (see fig. II.5). As Warburg himself explained, in employing this technique he had been inspired by the work of the English plant physiologist Horace Brown and his collaborator Francis Escombe.[27] Rotating sectors were standard instruments in the field of photophysics and, therefore, regularly used in the optical laboratory of the Berlin PTR, in which Warburg carried out many of his early experiments in photosynthesis.[28] Thus, it was not surprising that Warburg used – and indeed improved upon – this technique in his later work.

Inhibitors

Warburg was the first to use biological inhibitors as a means to find out more about the biochemical mechanisms of photosynthesis. For example, he investigated the effect of anaesthetics, such as the different urethanes, on the rate of photosynthetic assimilation. Warburg was also the first to study systematically how hydrogen cyanide affects the process. The rationale behind this technique was that Warburg already knew the inhibiting effect of these substances from his studies in respiration: urethanes were known to inhibit processes dependent on internal cell surfaces, while hydrogen cyanide blocked haemoglobin's site of oxygen binding (at the iron component). If the overall process of photosynthesis, or some of its different processes, were also inhibited by these substances, one could then conclude that certain causally relevant factors were inactivated by the inhibitors;

[27] See Brown & Escombe (1905), p. 38; Warburg acknowledges Brown's influence in Warburg (1919), p. 263.
[28] See Warburg (1919), pp. 235 and 255.

and one could then draw inferences on the properties of these factors and, hence, on the way the process functions.

3 Warburg's Early Photosynthesis Model

Warburg's first two articles were programmatically entitled: "On the rate of decomposition of the photochemical carbonic acid"; like most other researchers working at the time, Warburg took it for granted that the decomposition of carbonic acid was the source of oxygen in photosynthesis.[29] As Warburg stated in his synopsis of 1921, his superordinate research goal was to ascertain "by which means those substances that take part in the assimilation process are rendered reactive in living cells".[30] Thus, he confronted himself with the curious fact that, in the process of photosynthesis, one of the most stable chemical compounds, namely carbon dioxide, decomposes, although under normal circumstances (notably at room temperature) it is usually almost completely inert. Warburg's explanation was that this reactivity was, in short, achieved by the participating substances binding onto the surfaces of those solid cell constituents that contain heavy metals. Therefore, if these surfaces were destroyed, then the reaction sites were destroyed and, hence, photosynthesis was inhibited.[31] Warburg postulated that three different classes of reaction were involved:

(i) A primary photochemical process of light acting on a cell's pigments. The product of the process was a strong reducing agent, which Warburg called "the primary photochemical product" (PPP).

(ii) The formation of a carbonic acid derivative through a series of ordinary chemical reactions. This process required the involvement of heavy metals, which are embedded in the internal surfaces of the cell, and included the intermediate binding of carbonic acid to components of the cell. Thus, the process was surface dependent.

(iii) Secondary reactions in which the carbonic acid derivative reacts with the PPP, which would eventually lead to the release of oxygen and the synthesis of organic substances. These reactions were also thought to be surface-dependent chemical processes.

In sections 3 and 4, I shall present Warburg's main evidence for these hypotheses and then reconstruct the course of his argumentation.[32]

3.1 Experimental Findings and the Interpretations thereof

Carbon Dioxide Concentrations

After a detailed explanation of his new techniques, Warburg began his 1919 paper by re-examining the standard parameters of photosynthesis as investigated thus far by plant physiologists. (Note that none of the actors in Chapter I had even

[29] Warburg (1919) and Warburg (1920*b*). The original German title reads: *Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung.*

[30] See Warburg (1921), p. 354.

[31] See Warburg (1921), p. 354.

[32] On Warburg's first model of photosynthesis, see also Nickelsen (2007).

attempted to do this. However, even if they had considered this option, they would not have had the methods to do so.) The first theme Warburg revisited was the relationship between photosynthesis and the levels of carbon dioxide concentration, measured at high light intensities. His results are given in figure II.6. There were no surprises here. Warburg confirmed the findings of Frederick F. Blackman and his collaborators, who in 1905 had established the fundamental Law of Limiting Factors, a reformulation of Justus von Liebig's Law of the Minimum, stating that it is not the totality of resources that limits the rate of a chemical reaction (or of a physiological process such as growth) but the availability of the scarcest factor.[33] As Blackman had demonstrated, at low carbon dioxide concentrations the rate of photosynthesis increased in proportion to a rise in carbon dioxide concentrations. However, after a certain point, additional increases in carbon dioxide concentrations no longer promoted the rate of photosynthesis as efficiently as before, until the rate remained constant, notwithstanding any further increases in the gas. Like Blackman before him, Warburg concluded that, while in the first part of the curve carbon dioxide concentrations limited the rate of the process, in the second part of the curve some other limiting factor must have been present. Yet, Warburg gave the theme a new turn by proposing that, since light intensity and temperature were chosen favourably, the limiting factor in the second part of the curve had to be an additional substance, X, which would react with carbonic acid in the course of photosynthesis.[34] Substance X might possibly be a component of the green cells, Warburg hypothesised, alluding to Willstätter's discovery of the occurrence of this type of reaction.[35] Carbonic acid would react with substance X to make an unknown derivative, and only then could further reaction steps occur, leading to the release of oxygen. A graphical reconstruction of the sequence of events that Warburg proposed is shown in figure II.7.

Light Intensity

The second issue that Warburg re-examined was the relationship between photosynthesis and light intensity, measured at high carbon dioxide concentrations. Blackman, too, had studied this topic. The resulting curve, which again agreed with earlier studies, is reproduced in figure II.8: at low light intensities the rate of photosynthesis increased in proportion to the light, while this effect became less prominent at higher light intensities. After a certain point, the rate of photosynthesis reached a plateau and additional increases in light intensity were unable to promote the process any further. Again, the phenomenon itself was well known (although Warburg's new technique produced a slightly different curve), but Warburg proposed his own interpretation, at the same time underlining the similarity of this effect to the one described earlier in the paper:

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[33] See Blackman (1905). The English plant physiologist Frederick Frost Blackman was the first to investigate, together with various collaborators, the influence of certain parameters on the rate of photosynthesis, including light intensity, temperature and carbon dioxide concentrations.

[34] Warburg (1919), p. 253. English translation: "We can understand the shape of the curve if we take the rate of assimilation to be proportional to the concentration of carbonic acid and the concentration of a second substance, which reacts with the carbonic acid."

[35] Warburg (1919), p. 253; Warburg cites Willstätter & Stoll (1918), p. 172 and pp. 226ff., on this point.

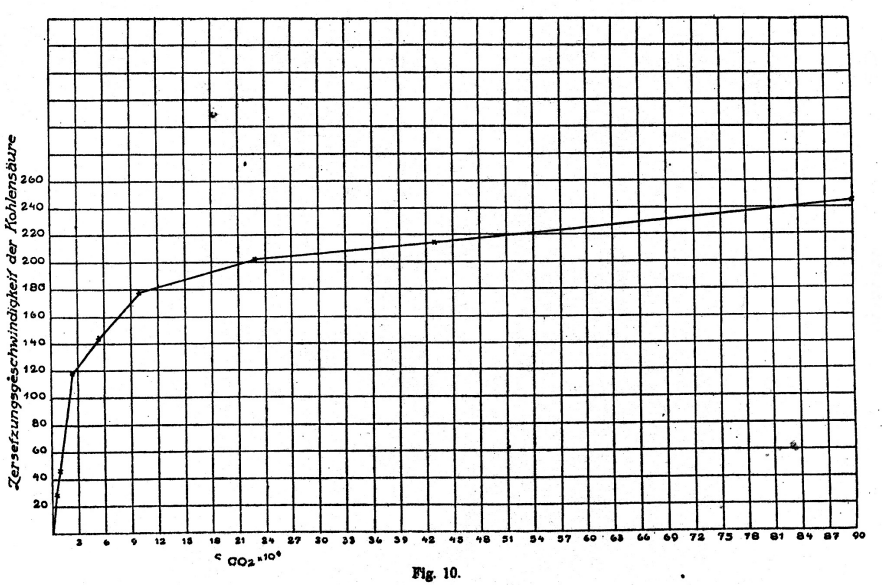

Figure II.6: The carbon dioxide curve of photosynthesis in Warburg (1919), p. 254, fig. 10. The vertical axis is labelled "Rate of carbonic acid decomposition" ("Zersetzungsgeschwindigkeit der Kohlensäure"); the horizontal axis is labelled "$c_{CO_2} \times 10^6$".

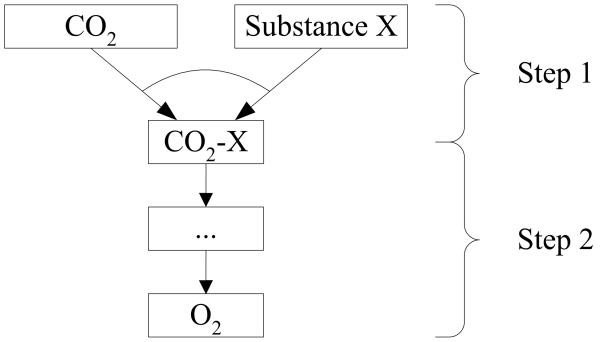

Figure II.7: A reconstructed model of Warburg's interpretation of the carbon dioxide curve. In the first part of the curve, CO_2 itself would be limiting the process, while a second substance, X, was thought to be the limiting factor in the second part of the curve, so that no additional increase in carbon dioxide concentrations would be able to promote the formation of oxygen any further. The formed complex of carbon dioxide and substance X (the "carbonic acid derivative") was assumed to undergo further reaction steps before oxygen could be released.

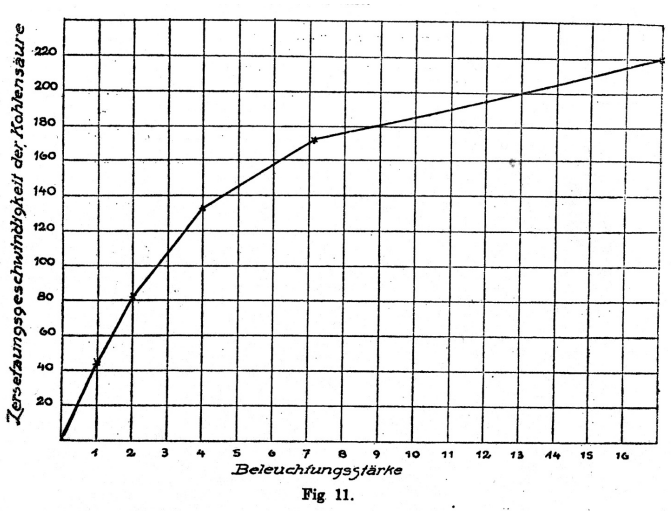

Figure II.8: The light curve of photosynthesis in Warburg (1919), p. 257, fig. 11. The vertical axis is labelled "Rate of carbonic acid decomposition" ("Zersetzungsgeschwindigkeit der Kohlensäure"); the horizontal axis is labelled "Light intensity" ("Beleuchtungsstärke").

> The appearance of the curve is very similar to the one that demonstrates the influence of different carbonic acid concentrations at constant light intensity; the "concentration of light energy" operates in this case like the concentration of a chemical substance. This concordance suggests that each light intensity corresponds to a specific concentration of a primary photochemical product, which, according to its concentration, would, in turn, be effective in a chemical reaction. The explanation of the shape of this curve would then have to be similar to the earlier one, by assuming that the rate of assimilation is in proportion to the concentration of the primary photochemical product and the concentration of a second substance, which reacts with this primary photochemical product.[36]

Thus, Warburg also thought that the light curve resulted from two different factors that influenced the rate of photosynthesis under different light conditions. Indeed, this time Warburg went even further, since he not only proposed two different *factors* but also two different *reactions* that would limit the whole process at low or high light intensities.[37] This was the first time that the shape of this curve, well known since the time of Blackman, had been explicitly interpreted in this way. If one follows Warburg's argument, a series of at least three reaction steps emerges (see fig. II.9): in the first stage light reacts with some other substance, Z, to form

[36] Warburg (1919), pp. 257–258.

[37] Warburg also interpreted the shape of the CO_2 curve to indicate that two different reactions were required to form the carbonic acid derivative. However, he did not elaborate on this point any further and dropped it completely in his 1921 article; therefore I have also omitted it from my discussion. All he says is that he assumed the existence of "two slowly proceeding and linked reactions; during these reactions carbonic acid is chemically altered through intermediary bonding to a cell constituent, while the end product of this process would be the photochemical acceptor of the carbonic acid assimilation". See Warburg (1920*b*), pp. 210–211.

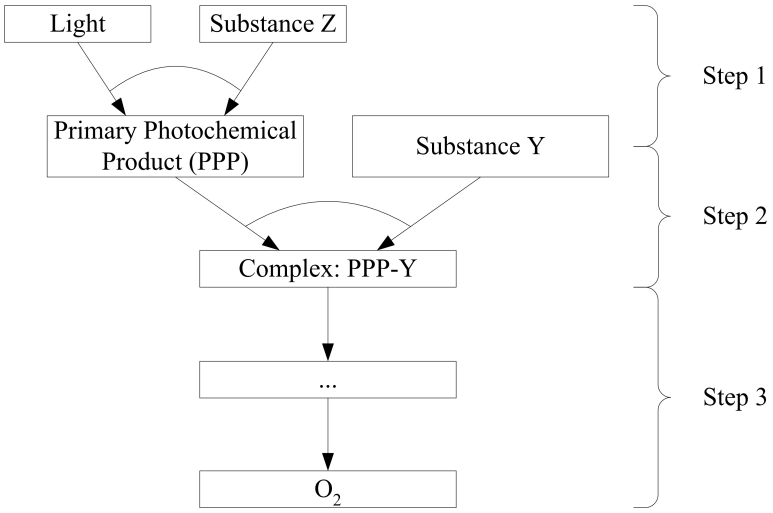

Figure II.9: A reconstructed model of Warburg's interpretation of the light curve. The first step consists of a primary photochemical reaction of light with substance Z, resulting in the primary photochemical product (PPP; Step 1). This product immediately undergoes a reaction with a second substance, Y, and a complex of PPP and Y is formed (PPP-Y; Step 2), which is then subject to further reaction steps leading to the release of oxygen (Step 3).

the PPP, which in the second stage reacts with another substance, Y, to further the process, before oxygen could be released in the final, third stage.

Temperature

Finally, Warburg also re-examined temperature, the third classic parameter of photosynthesis. At high concentrations of carbonic acid and at high light intensities, Warburg found, at the standard temperature interval between 15°C and 25°C, a temperature coefficient of about 2 (that is, with a rise in temperature of 10°C the reaction rate doubled), which was in agreement with the literature.[38] This indicated that under these conditions a thermochemical process was limiting the assimilation rate. At low carbonic acid concentrations and at high light intensities, Warburg found coefficients of 4 to 5, that is, an even stronger dependence on temperature; again, a thermochemical reaction was, presumably, a limiting factor – this, too, was not a new finding. And, finally, at low light intensities, Warburg confirmed "Blackman's important discovery", as he called it, of a coefficient approaching unity, which would mean that under these conditions the rate of photosynthesis is governed by a process that is practically temperature independent: a photochemical reaction was the obvious answer.[39] In his 1921 article, however, Warburg slightly revised this last result by presenting evidence which showed that at low light intensities the coefficient was *negative*, that is, the rate of the process rose as the

[38] Warburg (1919), p. 258.
[39] Warburg (1919), p. 259.

temperature decreased. This, Warburg argued, indicated that in this process high energy substances, such as PPPs, were the limiting factor.[40]

Intermittent Illumination

The next subject that Warburg turned to was new: the effect of exposing photosynthesising cells to alternating dark and light periods. In order to investigate this effect, Warburg used the aforementioned rotating sectors (see fig. II.5, p. 77): a disc with one or more sections was placed between the light source and the algae, so that part of the light could be screened off. Warburg chose the size of the sections in such a way that in the course of one rotation half the light would be screened off. Therefore, in two experiments of the same duration, one with rotating sectors, the other without, the former would receive only half the light energy of the latter. Thus, Warburg did not compare the effects of continuous and intermittent illumination with experiments of like duration but with experiments of like light exposure time. In doing so, he found that at high light intensities a certain amount of energy was able to decompose more carbonic acid at intermittent illumination than at continuous illumination. The increase in efficiency depended on the alternation rate between light and dark periods: at a rate of 8,000 alternations per minute efficiency increased by almost 100 per cent, while at a rate of four alternations per minute an increase of only 10 per cent was achieved. At low light intensities no differences in efficiency were observed. From these findings, Warburg concluded the following:

> If a certain amount of energy, which, when alternated with dark periods of equal length, is 100% more efficient than the same amount of energy at continuous illumination, we might as well say: in a time interval that is long compared with the length of one period at intermittent or continuous illumination the same amount of carbonic acid is broken down, that is, the average assimilation rate is the same using both types of illumination.[41]

Warburg proposed two alternative explanations: either decomposition of carbonic acid continued to occur during the dark periods at the same rate as before, possibly because of some sort of energy storage; or decomposition was interrupted during dark periods, and then resumed during periods of light at double the rate. Warburg preferred the latter interpretation, and suggested that, while decomposition itself stopped when the source of light was interrupted, other processes would continue until an equilibrium state had been reached (which at continuous illumination would never be attained). Warburg also assumed that during these "dark" processes a substance was formed that could be decomposed by light energy. As a higher concentration of decomposable substance would be available after a dark period, light could then act more efficiently – supposing that light of sufficient intensity was available; at low light intensities the products produced during the dark periods would not be properly processed. This interpretation perfectly matched Warburg's theory on the light intensity curve: the light-dependent reaction – the primary photochemical process – provided only part of the necessary starting materials for the eventual release of oxygen; the other component was

[40] Warburg (1921), p. 355.
[41] Warburg (1919), pp. 262–263.

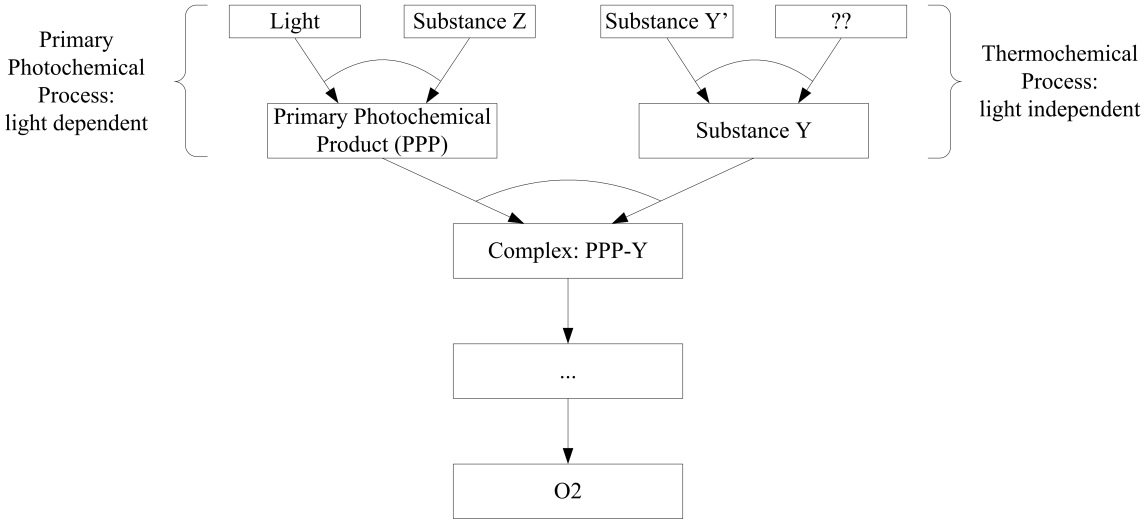

Figure II.10: The extended model of Warburg's interpretation of the light curve: whereas the PPP is formed during a light-dependent process, substance Y is produced during a light-independent series of reactions. The former limits the rate of photosynthesis at low light intensities, the latter at high light intensities.

supposed to be an additional substance, Y, which had to react with the PPP (see above and fig. II.9). In addition, Warburg now assumed that substance Y was derived from a precursor substance, Y', by way of light-independent chemical reactions. With the resumption of light after a dark period, therefore, the PPP would encounter increased concentrations of Y and the process would thus proceed at a higher rate (see fig. II.10 for the extended model).

Anaesthetics

The effect of inhibiting substances, especially anaesthetics, on photosynthesis played an important role in Warburg's reasoning (see section 2); he predominantly investigated the effect of urethanes, in particular phenylurethane, on the process. As was well known at the time, photosynthetic assimilation could be reversibly inhibited by these substances. Warburg confirmed this general finding for green algae and extended it to his conclusion that photosynthesis was far more sensitive in this respect than, for example, respiration.[42] He interpreted this finding following the general mechanism of anaesthesia:

> Taking into account that the effect of anaesthetics is due to changes in the boundary layers, one must conclude that the slightest changes in these layers thus inhibits the process of [photosynthetic] assimilation. This agrees with the experience that, in contrast to other life processes, as, for example, respiration

[42] Warburg (1919), p. 265: "It can be inferred from the table [of the experimental data] that concentrations that only just inhibit the [photosynthetic] assimilation of the algae are about twenty times lower than concentrations that inhibit respiration. [...] If one were to arrange the life processes according to their sensitivity to the inhibiting effects of anaesthetics, assimilation would be in first place."

> and fermentation, the slightest mechanical change to the cell structure will suspend [photosynthetic] assimilation.[43]

This interpretation matched Warburg's finding that the inhibiting effect of an anaesthetic substance was stronger, the higher its adsorptive capacity, that is, its tendency to adhere to surfaces.[44] Since the inhibiting effects were observed under all circumstances – that is, at low and at high light intensities as well as at different carbon dioxide concentrations[45] – Warburg concluded that all the reactions that limited the rate of the process under different conditions were surface dependent. That photosynthesis is sensitive to anaesthetics at high light intensities and low carbon dioxide concentrations, for example, demonstrated that the limiting process under these conditions (which he considered to be the bonding of carbonic acid to an unknown substance, X) was to be seen as a reaction that took place on the cell's internal surfaces, presumably on the surface of the membranes.[46] The same applied to the limiting process at low light intensities and at high carbon dioxide concentrations, which also proved sensitive to anaesthetics: according to Warburg, the limiting process under these conditions was the light-dependent stage; however, since the absorption of light itself was surely not sensitive to anaesthetics, Warburg concluded that the limiting process here must also involve a secondary (although indispensable) surface-sensitive reaction.[47] This corresponded well to his assumption that a primary photochemical step – the absorption of light by substance Z – was followed by a subsequent interaction of the resulting product with another substance, Y (see fig. II.8).

Hydrogen Cyanide

In addition, Warburg examined the influence of hydrogen cyanide, another substance with inhibiting effects, which acted in a fundamentally different way to the urethanes. Warburg demonstrated that even at very low concentrations of this substance – such as by an n/10,000 hydrogen cyanide solution – assimilation was reversibly inhibited.[48] By contrast, respiration was not even inhibited by an n/100 solution of hydrogen cyanide, that is, at a 100-fold higher concentration. However, this strong inhibition of photosynthesis could only be observed at high light intensities, whereas at low light intensities it was far less obvious. Thus, Warburg concluded, "the limiting process at high light intensities is sensitive to hydrogen cyanide, while the limiting process at low light intensities is insensitive [to hydrogen cyanide]".[49]

In order to explain this finding, Warburg introduced the notion of the gas exchange equilibrium, that is, the state of a slightly illuminated cell in which assimilation or oxygen production and respiration or oxygen consumption equal

[43] Warburg (1919), pp. 265–266. Note that Warburg used the term *Grenzschichten* which was translated as "boundary layers".

[44] See Warburg (1920*b*), pp. 196–197.

[45] The effect at high light intensities and at low carbon dioxide concentrations was only demonstrated in the second article; see Warburg (1920*b*), p. 197.

[46] See Warburg (1920*b*), p. 199.

[47] See Warburg (1919), p. 266.

[48] Loc. cit.

[49] Loc. cit.

each other; consequently, at this point no gas exchange is measurable. Today this is known as the "compensation point" of photosynthesis. Now, when Warburg tested the effects of hydrogen cyanide, he found that, below the compensation point, when respiration was predominant, oxygen production in the course of photosynthesis was being inhibited in proportion to the concentration of hydrogen cyanide. However, from the compensation point onwards, there was no increase in effect when more hydrogen cyanide was added, "which means", Warburg argued, "that the influence of illumination on the respired oxygen is only slightly inhibited, even by large amounts of hydrogen cyanide".[50] He explained this in more detail:

> The data listed in Table IXa demonstrate that n/500 hydrogen cyanide solutions completely inhibit the release of oxygen from carbonic acid [in photosynthesis]; even at high light intensities of 19,000 lux, the cell is no longer able to develop any positive pressure. However, a certain [low] amount of illumination will split the respiration products and release oxygen in cells treated with hydrogen cyanide at the same rate as in cells without hydrogen cyanide. Thus high concentrations of hydrogen cyanide have no effect on the photochemical reaction mechanism – as can be seen from the effect on the oxygen that was bonded in the course of respiration – but they inhibit the ability of carbonic acid to undergo photochemical reactions.[51]

Warburg's point was that illuminated cells, under any conditions, would release some oxygen, which had not, however, been produced by photosynthesis but by the photochemical effects on other substances within the cell – for example, by the effect of light on the products of respiration, which Warburg identified as being mainly, although not exclusively, carbon dioxide. Since this photochemical splitting of molecules still went on even at high concentrations of hydrogen cyanide, Warburg argued that the inhibiting effect of this substance on assimilation had to be caused by the blocking of other processes. Thus, Warburg suggested that hydrogen cyanide inhibited the ability of carbonic acid "to undergo photochemical reactions" (see quotation above). This corresponded to Warburg's assumption that carbonic acid had to bind to another substance, X, before the resulting derivative could be decomposed. It was exactly this binding process that Warburg thought would be inhibited by hydrogen cyanide; and since it was known from other contexts that (1) hydrogen cyanide mainly acted by inactivating necessary heavy metals and that (2) these heavy metals were usually part of the catalysing enzyme, Warburg inferred that the reaction in question was an enzyme-catalysed reaction requiring the involvement of heavy metals.

Photochemical Induction

The next finding, presented in Warburg's second article of 1920, was the phenomenon of "photochemical induction". The principle effect had first been observed in the photochemical reaction between chlorine and hydrogen: if this mixture was irradiated, hydrochloric acid was formed; however, the rate of this reaction was initially slow, gradually accelerating to a constant final value. As Warburg explained, this delay had been shown by Walther Nernst to be primarily caused

[50] Warburg (1920*b*), p. 199.
[51] Warburg (1920*b*), pp. 203–204.

by secondary reactions of this chain reaction process rather than by the primary photochemical reaction.[52] A similar phenomenon, Warburg argued, could also be observed in photosynthesis, when studied under intermittent illumination:

> If one switches to long periods [of darkness and light] and, in addition, prolongs the dark periods in comparison to the light periods, it turns out that a certain amount of radiation, which, when alternated with dark periods, breaks down less carbonic acid than the same amount under continuous illumination.[53]

Only after some minutes of illumination, Warburg reported, would the usual constant value be reached. As he demonstrated with his data, extending the dark periods by up to five minutes resulted in a decrease in efficiency of the following light period of 70 to 80 per cent compared with the efficiency of the same radiation without any dark periods. Thus, Warburg concluded that the assimilation rate after dark periods rose only gradually. In order to be able to measure this increase manometrically, Warburg first exposed his algae to a five-minute dark period; then he had the same algae irradiated for 0.5 to three minutes. However, since it took some time before newly formed oxygen could be detected by manometric methods, Warburg darkened the cells again and only took the reading after a few more dark minutes, that is, once a constant value had been reached. Furthermore, Warburg worked with very thick cell suspensions. Using this set-up, he found that at 25°C a constant rate of assimilation was only reached after a time lag of two minutes. However, this was only the case at high light intensities, as he could not demonstrate any such delay at low light intensities. In this respect Warburg's observations differed significantly from the usual induction phenomenon known to photochemistry in general: whereas the induction period of the chlorine-hydrogen reaction was shorter the higher the light intensity, Warburg observed the reverse in photosynthesis. Thus, Warburg concluded, the explanation for the two phenomena had to be different as well. He suggested the following:

> This phenomenon [i.e. the induction period in photosynthesis] cannot be interpreted by assuming that during the dark periods substances accumulate that would immediately react with the oxygen that is formed on illumination, so to say, *in statu nascendi*; in this case the induction period should be longer, the lower the intensity of illumination, while in actual fact the opposite can be observed. Thus, it rather follows from the observations that 1) no oxygen is released in the course of the primary process and 2) no substances are formed in the course of the primary process that would spontaneously (in dark reactions) give rise to oxygen. [...] Points 1 and 2 are all that can safely be said about the primary process; both make it very unlikely that the primary process concerns the carbonic acid molecule.[54]

Oxygen

Warburg finally investigated the influence of different oxygen concentrations on photosynthesis. As was known, for example from Willstätter's experiments, for

[52] See Warburg (1920*b*), p. 189.
[53] Warburg (1919), pp. 265–266.
[54] Warburg (1920*b*), pp. 208–209.

photosynthesis to occur a certain minimum level of oxygen needed to be present – according to Willstätter an amount of less than 1/1000 atmosphere (atm). Starting from this, Warburg studied the influence of higher oxygen concentrations (1/50 to 1 atm) at high light intensities and found that under these conditions the photosynthesis rate decreased as oxygen partial pressure increased; however, the effect diminished when oxygen partial pressures approached 1 atm.

Warburg considered two possible explanations: either the oxygen re-oxidised the intermediate products of photosynthesis to carbon dioxide, so that, in effect, the end product stage would never be reached or the oxygen competed with the (modified) carbonic acid as an acceptor of the energy transferred by the PPP and by this means prevented the process from being completed. Warburg, of course, favoured the latter explanation, as it was compatible with his model of the mechanism.

3.2 Photosynthesis Framed as Photolysis

Warburg integrated all these findings into a comprehensive interpretation of the mechanism of photosynthesis, which is reconstructed in graphical form in figure II.11. Warburg considered photosynthesis as a complex form of "photolysis", that is, "light splitting" – a concept that had been introduced by his father Emil in the course of his studies in general photochemistry. The substances that were decomposed by photolysis were called "photolytes" (both terms were clearly derived from the words "electrolysis" and "electrolytes"). In all such reactions, Warburg explained, one had to distinguish between the primary and secondary processes: "The primary reaction always involves a change in the [light] absorbing molecule, while the secondary reactions take place between the photochemical primary products or between these and other constituents of the photolyte."[55] The latter, that is, the constituents of the photolyte which react with PPPs, would be called "acceptors". However, as Warburg stressed, photosynthetic assimilation was "not a simple photolysis of carbonic acid":[56]

> The primary photochemical process, during which oxygen is *not* released, affects the chlorophyll molecule and leads to the formation of the primary photochemical product. The rate of the formation of the primary photochemical product is in proportion to the amount of radiation absorbed per time unit. The concentration of the primary photochemical product is determined both by the rates of its formation and its consumption. The primary photochemical product reacts with the acceptor during secondary reactions.
>
> The acceptor is not carbonic acid but a derivative of carbonic acid, which is formed in the cell by a chain of chemical reactions. Thus, there is a third class of reactions in the cell, in addition to the primary photochemical process and the secondary reactions: namely, acceptor formation. Acceptor formation is a sequence of spontaneous reactions, which, without illumination, would quickly come to rest, due to the accumulation of end products. On illumination, however, the end products – the acceptors – are consumed during the secondary reaction, which destabilises the dark equilibrium.
>
> Both the reactions that lead to the formation of the acceptor and the reaction between the acceptor and the primary photochemical product are

[55] Warburg (1920*b*), p. 206.
[56] Warburg (1920*b*), p. 206.

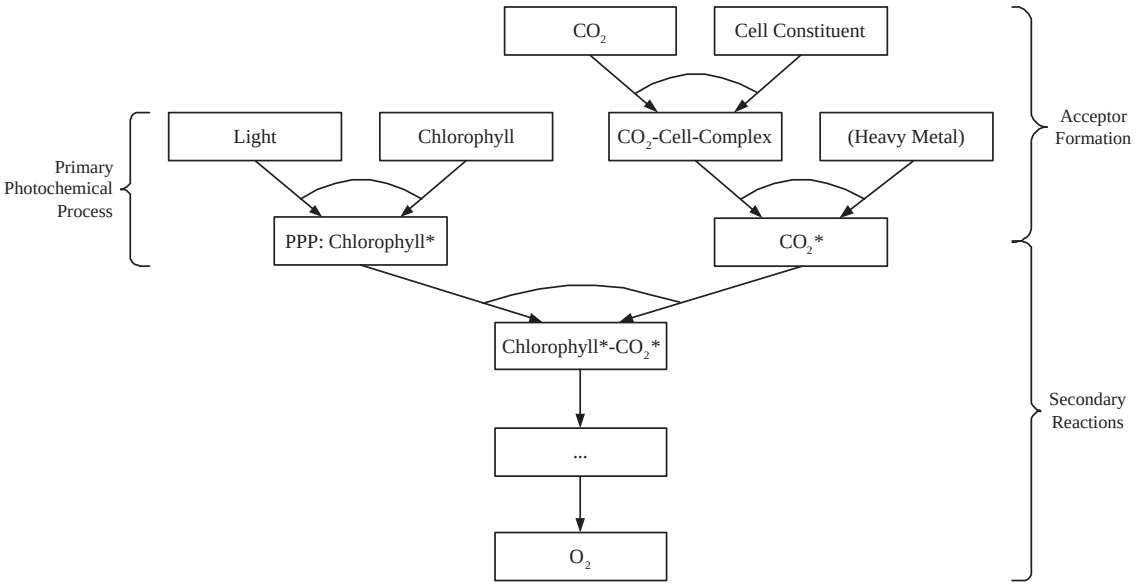

Figure II.11: A reconstruction of Warburg's photosynthesis model.

> surface-dependent and, thus, they are extremely sensitive to changes in the surface environment.
>
> In contrast to the secondary reaction, the formation of the acceptor is inhibited by small amounts of hydrogen cyanide. Since the action of hydrogen cyanide probably consists of the transformation of heavy metals from an active form into an inactive complex compound, one should consider the involvement of heavy metals in the process of acceptor formation.[57]

This was the core of Warburg's photosynthesis model, to which he made some additions in 1921. The full sequence of reactions, as Warburg conceptualised them, is reconstructed in graph form in figure II.11. In order to clarify matters, I shall briefly comment on Warburg's own statement and summarise the additions, using figure II.11 as a reference. According to Warburg, the primary process was the most elusive reaction of the whole mechanism of photosynthesis. Not much could be said about it; the only safe conclusions Warburg felt entitled to draw were that this process did not yet give rise to oxygen and that it involved a change in a light-absorbing molecule. In his 1920 article Warburg identified this molecule as chlorophyll (see the quotation above), but in 1921 he qualified this statement and spoke about the cell's pigments in general, that is, the two kinds of chlorophyll (*a* and *b*), the xanthophylls and the carotenes.[58] (To simplify matters, the figure II.11 includes only the chlorophyll molecule.) On absorbing light energy, the short-lived PPP is formed, which in 1921 Warburg assumed to be the "isomers of these [light absorbing] pigments, enriched in energy by hν".[59] The higher energy level of chlorophyll in this activated state is indicated in the figure by an asterisk (*).

[57] Loc. cit.
[58] See Warburg (1921), p. 354.
[59] Loc. cit.

At the same time, Warburg also held that a second sequence of purely chemical reactions – acceptor formation, as he called it – was necessary if photosynthesis were to continue to occur.[60] Because of this chain of reactions, Warburg argued, photosynthesis was highly temperature dependent at high light intensities, that is, when there was plenty of light energy available. In his 1921 article, Warburg used the term "Blackman reaction" for the first time to describe the process that limited photosynthesis under these conditions; it was to become the standard term for this stage of photosynthesis.[61] According to Warburg, it was this class of reactions (which formed an activated carbonic acid derivative) that made carbonic acid susceptible to cleavage. The complete series of reactions was yet unknown, but Warburg considered that at least two steps were necessary: the intermediate binding of carbonic acid to some cell constituent and, subsequently, a reaction step that somehow modified the bonded carbonic acid. Since this partial process proved highly sensitive to hydrogen cyanide, Warburg assumed that, in the second step, a heavy metal was involved (presumably iron). This would contribute to converting the carbonic acid into its activated derivative (the activation is indicated in figure II.11 by an asterisk [*]). This process was also shown to be surface dependent because of its high sensitivity to anaesthetic substances such as urethanes. In short, acceptor formation was, in Warburg's model, thought to be the result of the catalytic action of an enzyme that contained heavy metals and occurred on internal surfaces. The end product of this reaction was a reactive carbonic acid derivative.

Finally, the PPP and the acceptor – that is, the activated pigment and the carbonic acid derivative – were assumed to interact with each other during secondary reactions: the photochemical acceptor was reduced by the reducing agent, that is, the carbonic acid derivative was reduced by light-activated pigments. Warburg did not go into much detail here, except to characterise these reactions again as surface-dependent, purely chemical processes. This was inferred from the fact that even at low light intensities, when assimilation could still be increased in proportion to the light intensity, the process was sensitive to surface-active substances, which, Warburg argued, could not be ascribed to light absorption processes alone. Thus, in addition to light absorption, secondary chemical reactions were also limiting the rate of photosynthesis at low light intensities, while at high light intensities the Blackman reaction, that is, acceptor formation, was thought to be the limiting factor.[62]

4 The Efficiency of the Process

To complement this model of the photosynthesis mechanism, in 1922 and 1923 Warburg carried out an investigation into the efficiency of the process, which he co-

[60] Again, it should be noted that the current usage of "acceptor" does not correspond to Warburg's notion of the term.

[61] See Warburg (1921), p. 355.

[62] Note that Warburg changed his mind in 1925 and adopted Willstätter and Stoll's notion of the Blackman Reaction. They believed that Warburg's "secondary reactions", that is, the reduction of the carbonic acid derivative in the chlorophyll complex, was the Blackman Reaction, which limited the rate of photosynthesis at high light intensities. See Warburg (1925).

authored with Erwin Negelein.[63] The question they hoped to answer was: "Which fraction of the absorbed radiation energy can be transformed into chemical energy in the process of carbonic acid assimilation?". This was, as the authors (somewhat exaggeratingly) noted, "a question which has frequently been debated, but so far still not answered".[64] If the absorbed radiation energy is called E and the chemical work accomplished at the same time is called U, then Warburg and Negelein were looking for the quotient U/E. This quotient had been introduced in 1920 by Emil Warburg, who had defined it as a "specific photochemical effect" (*spezifische photochemische Wirkung*), abbreviated to φ, which denoted the chemical work effected by one calorie of absorbed radiation.[65] It was known to increase at diminishing light intensities, that is, at low light intensities photochemical reactions tended to be more efficient, until a maximum value was reached close to zero light intensity. It was precisely this limiting case, called φ_0, in which Warburg and Negelein were interested: the "photochemical yield" (*photochemische Ausbeute*) of photosynthesis.[66]

In addition to the theoretical concepts that the authors clearly borrowed from Emil Warburg, they also made use of the latter's facilities. As Warburg and Negelein acknowledged in their article, all the relevant experiments were carried out in Emil Warburg's laboratory at the PTR (the very laboratory in which Otto Warburg had learned how to employ bolometry, which was indispensable for this kind of experiment), where they used the institute's high-quality area bolometer.[67] In their experiments, Warburg and Negelein exposed *Chlorella* algae to light of wavelengths between 570 and 645 nm, that is, from yellow to red light. In order to get a reliable value for the amount of absorbed energy, E, Warburg and Negelein used very thick algae suspensions, so that practically all the incident light on the solution was absorbed. By contrast, U was measured manometrically, with the measured oxygen release taken as the indicator value.

The results of this study included the important finding that the efficiency of photosynthesis was highly dependent on the conditions under which the algae had been cultivated: the highest efficiency was achieved with cells that had been transferred to low light intensities after having been grown for some time in high light intensities. Furthermore, as one would expect, taking Warburg's model of the process as the starting point, surface-active substances were found to reduce photosynthetic efficiency in proportion to their adsorptive capacities. This indicated that the underlying process, which Warburg and Negelein characterised as follows, was surface dependent:

> The pigments of the chromatophore [i.e. chloroplast] are embedded into the colourless framework of the chromatophore, forming a solid adsorbent. Wherever this coloured adsorbent encounters the colourless, watery content of the chromatophore, carbonic acid is adsorbed in a form that is yet unknown. This explains the fact that dissolved or colloidally distributed chromatophore pigments are unable to split carbonic acid on illumination. All attempts to

[63] Warburg & Negelein (1922) and Warburg & Negelein (1923).
[64] Warburg & Negelein (1922), p. 235.
[65] See Warburg (1920*a*), p. 54.
[66] Warburg & Negelein (1923), p. 205.
[67] Warburg & Negelein (1922), p. 236.

> reduce carbonic acid by means of pigments that were dissociated from the chromatophore have so far yielded negative results.[68]

The efficiency measurements themselves revealed that, on average, an extremely high percentage of between 60 and 70 per cent of the absorbed radiation energy could be transformed into chemical energy. Note that until then, the highest efficiency that had ever been measured for chemical reactions (Warburg's father, for example, had measured the efficiency of ozone formation) had been one of 50 per cent! Warburg and Negelein furthermore drew attention to the fact that, due to their experimental set-up and measuring process, these values should be considered too low, and that the actual efficiency might be even higher.[69] These were spectacular findings. Although the maximum quantum yield of photosynthesis had occasionally been discussed in the year before Warburg and Negelein turned to the subject, it was far from the "frequently debated" issue that the authors has claimed it to be in their introduction. Measuring quantum yields certainly occupied the attention of photochemists; yet very few people had so far tried to transfer this approach to the study of photosynthesis. The standard estimation of the efficiency of photosynthesis was provided in 1905 by the aforementioned English plant physiologists Brown and Escombe, who found a maximum efficiency of photosynthesis of 6 per cent – which was clearly much lower than Warburg and Negelein's values. As was mentioned earlier, Warburg and Negelein acknowledged the study of Brown & Escombe (1905) as a source of inspiration for their own work, particularly in terms of the use of rotating sectors; however, in most other respects, Warburg and Negelein sharply criticised the earlier efficiency experiments and found Brown and Escombe's results invalid, since they believed that the whole set-up was flawed and inappropriate.[70] Warburg and Negelein's main point was that Brown and Escombe had used whole leaves and measured light absorbance by observing the weakening of light passing through the leaf. Warburg and Negelein rightly argued that a large part of the issuing light would be scattered by the leaf and would, therefore, remain undetected by the instrument. Therefore, Brown and Escombe's value could not be considered reliable.

The efficiency of photosynthesis was particularly interesting in view of the ongoing search for the underlying mechanism. A simple calculation revealed that reducing one molecule of carbonic acid to the level of carbohydrates required, at the very least, an energy input of 112.3 kilocalories (kcal). From this it followed that, on average, the carbonic acid had to interact with at least three pigment molecules, if each of them absorbed one red light quantum with an average energy

[68] Warburg & Negelein (1922), p. 244.

[69] Warburg & Negelein (1922), p. 244. In their second publication of 1923, the average value of 1922 (70 per cent) was slightly reduced to an average (in red light) of 59 per cent efficiency, while the maximum value they had been able to achieve was 63.5 per cent efficiency. This was due to a change in procedure: while in 1922, Warburg and Negelein had determined φ_0 by extrapolating from values at higher light intensities, in 1923 they reconsidered this procedure, since, as they conceded, it was not known which curve the extrapolation should be made to follow. Instead, they measured the efficiency in the lowest possible light intensities, and when no significant increase in value was found, they assumed that this value was the limiting case. See Warburg & Negelein (1923), p. 205.

[70] See Warburg & Negelein (1923), pp. 192–193.

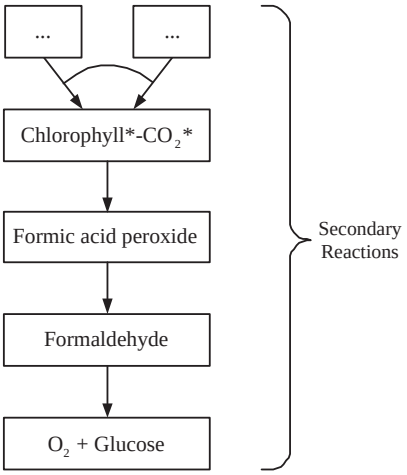

Figure II.12: The extension of Warburg's photosynthesis model by a possible pathway leading from the carbonic acid derivative to oxygen and glucose (only the secondary reactions have been included).

of 49 kcal each. Although Warburg and Negelein did not yet dare, in 1922, to draw any concrete inferences from their finding, they did emphasise that in view of the high overall efficiency of the process, the reduction of carbonic acid had to be straightforward, that is, without the inclusion of any intermediate reactions that would require additional energy. Possibly, as Willstätter had suggested in 1918, a formic acid peroxide was formed, which then, via a formaldehyde stage, would yield glucose in a condensation reaction.[71] The corresponding extension to Warburg's photosynthesis model is shown in figure II.12.

These general findings were complemented in 1923 by an investigation into the influence of different wavelengths on the efficiency of photosynthesis.[72] Warburg and Negelein's most important finding was that φ_0 decreased as the wavelength diminished, that is, the photochemical yield was lower at shorter wavelengths than at longer wavelengths. This finding was in agreement with quantum theory, in particular with Einstein's Law of Photochemical Equivalence, which predicted exactly that. It also concurred with Emil Warburg's measurements of the photochemical yield in the photolysis of hydrobromic and hydroiodic acids. Warburg and Negelein calculated that approximately four light quanta would be required if the algae were illuminated with red or yellow light, and about five quanta if they were illuminated with blue, to decompose one molecule of carbonic acid. These results were regarded as the authoritative answer to this question for the next twenty years or so; and it was these figures that sparked off the vigorous controversy on quantum yields and efficiencies between Warburg and almost the entire scientific community. This controversy is the subject of Chapter IV.

[71] Warburg & Negelein (1922), p. 249. Note also that Warburg favoured the standard formaldehyde module of carbohydrate synthesis, which was discussed in Chapter I.

[72] Warburg & Negelein (1923). Warburg and Negelein investigated the process at 610 to 690 nm (red), 578 nm (yellow), 546 nm (green) and 436 nm (blue).

As a closing remark to this description of Warburg's early photosynthesis work, I would like to draw attention to the following three points:

(i) Warburg explicitly adopted theoretical concepts – photolysis, the photolyte and the photochemical yield – that his father had used and developed before him;

(ii) Warburg undertook experiments along the lines of his father's earlier work and stressed that his findings concurred with his father's results.

(iii) Warburg carried out much of his research work on photosynthesis in his father's laboratory, where he used the PTR's optical infrastructure.

In view of these observations, I believe that the connection between Otto Warburg's research and the studies of his father Emil deserves special attention.

5 Father, Son and Photosynthesis

5.1 Emil Warburg and Photochemistry

The radiation laboratory of the PTR, to which Emil Warburg was appointed president in 1905, had a renowned history and a high international reputation.[73] One of the PTR's primary concerns around 1900 was the investigation of different light units, particularly the problem of defining a common standard measure. To this end, the PTR had first to develop new high-precision optical instruments, such as bolometers and other devices for measuring light absorption, as well as rotating sectors for working with varied light intensities and standardised light sources.[74] It was in this excellently equipped laboratory that Otto Lummer and his collaborators started to research black body radiation and carried out the measurements that eventually brought Max Planck to advance the existence of a universal energy constant. However, after Lummer left the PTR in 1904 to take up a professorship at the University of Wrocław (then Breslau), this instrumentation fell into disuse.

The revival of the radiation laboratory is today considered one of Emil Warburg's most notable achievements as President of the PTR. Shortly after having taken up this position, Emil Warburg inaugurated studies in photochemistry at the PTR; he chose to investigate the energetics of photochemical processes starting in 1907, while after 1911 he made it the focus of his work (between 1911 and 1919, he published nine substantial articles on photochemistry, a subject that clearly dominated the latter part of his career).[75] In contrast to the earlier methods of measuring radiation, which for the most part had yielded only qualitative results, Emil Warburg wanted to explain the photochemical energy conversion in quantitative terms. This ambition was not least sparked by Einstein's seminal publication on the light quantum hypothesis (1905), in which Einstein had postulated that: (1) radiation of the frequency ν will always be absorbed in discrete quanta of the amount h (Planck's constant) times ν; and that (2) every particle that absorbs light quanta of this type will be either split or modified in other

[73] See Cahan (1989) for an account of the PTR's history, 1871-1918.
[74] Brodhun (1913) provides a detailed review of the PTR's activities in optics.
[75] On Emil Warburg's photochemical work, see, e.g., Franck (1926) and Franck (1931).

ways.[76] Although this hypothesis could explain why the course of photochemical reactions was influenced by the colour of light, it failed to elucidate the existence of the so-called intensity thresholds which occurred in certain types of photochemical reactions. Einstein himself worked on this problem in 1909, when Emil Warburg was already well on his way to establishing the energy balance of photochemical reactions from a theoretical point of view.[77] Emil Warburg even devised a new type of bolometer for this project – the vacuum bolometer – which allowed for more precise measurements to be taken.[78] By 1911, Emil Warburg had also begun to tackle the question experimentally.[79]

Although the role of light in chemical processes had been much debated in the nineteenth century, the quantitative analysis of these phenomena had remained a problem.[80] On the one hand, methodical difficulties impeded the research, since photometry was still in its infancy. On the other hand, it was not at all clear why photochemical processes were not primarily influenced by the intensity of light, as theory predicted, but by the light's wavelength. Einstein summarised this inconsistency in 1909 as follows: "Why does the occurrence of a certain photochemical reaction only depend on the colour and not on the intensity of the light? Why are rays of shorter wavelengths generally more chemically effective than those of longer wavelengths?"[81] It did not take Einstein long to answer these questions, and they aroused considerable attention. As the editors of the fourth volume of *The Collected Papers of Albert Einstein* put it: "The derivation of the law of photochemical equivalence from purely thermodynamic considerations was, arguably, Einstein's major scientific contribution in the years 1912-1914."[82]

Einstein and Emil Warburg, who were approximately one generation apart, met at the first Solvay Conference (held in Brussels, Belgium) in November 1911, where they discussed photochemistry. It was not least this discussion that brought Einstein to formulate the well-known Law of Photochemical Equivalence.[83] One

[76] See Einstein (1905) for the original publication; the paper was reprinted (together with a short introduction and additional notes) in Vol. 2 of the *Collected Papers* edition; see Stachel, Cassidy, Renn & Schulmann (1989), pp. 134–169.

[77] See, e.g., Klein, Kox, Renn & Schulmann (1995), p. 110f.

[78] See, e.g., Warburg et al. (1907) and Warburg (1909).

[79] See, e.g., Warburg (1912) and Warburg (1913).

[80] See the introduction to Einstein's paper in Klein et al. (1995), pp. 109ff.; for a historical review of the problem, see Warburg (1917). See also Boberlin (1993) for an account of the nineteenth-century beginnings of quantitative photochemistry.

[81] Cited in Klein et al. (1995), pp. 109–110. Original German quotation in Einstein (1909), p. 490.

[82] See Klein et al. (1995), introduction, p. xiv. For Einstein's first paper on the subject, see Einstein (1912*b*), which is reprinted in Klein et al. (1995), Doc. 2, pp. 115–121. A short supplement, which gave the subject an additional turn, followed his 1912 article on the Law of Photochemical Equivalence; see Einstein (1912*a*), which is reprinted in Klein et al. (1995), Doc. 5, pp. 166–170. Both were combined in a lecture that Einstein gave to the Société Française de Physique in 1913; see Einstein (1913), which is reprinted in Klein et al. (1995), Doc. 12, pp. 287–293. Einstein also discussed the Law of Photochemical Equivalence in the physics seminar he held in the winter semester of 1912/13 and the summer semester of 1913; see Klein et al. (1995), p. 109 and Appendix A. For a detailed study of Einstein's work on photodecomposition, see Bergia & Navarro (1988).

[83] For details, see Klein et al. (1995), pp. 110–111. See also the letter from Einstein to Heinrich Zangger, dated 20 November 1911, and published in Klein, Kox & Schulmann (1993), Doc. 309,

consequence of this law was the fact that all photochemical reactions would then require the absorption of one light quantum per "photolyte" molecule, that is, per molecule that was able to undergo photochemical cleavage.[84] It was Emil Warburg who took up the task of providing experimental evidence for this law, which proved far more difficult than expected; however, in the end Emil Warburg's research into the photolysis of hydrogen bromide and cyclohexane (*Hexahydrobenzol*) proved fairly satisfying in this respect.[85]

After Einstein moved to Berlin in 1914, he was in close contact with Emil Warburg.[86] In 1915, for example, Einstein worked in the PTR's laboratory – although not, as Emil Warburg might have preferred, with him in the radiation laboratory, but with the Dutch physicist Wander de Haas, who was researching the gyromagnetic effect in metals at the time. Einstein and Warburg also met privately: Einstein was a regular visitor to the Warburg household; he became acquainted with Emil Warburg's wife and presumably also heard of Warburg's son Otto, although they only met after 1918.[87] However, thereafter the two remained in touch. It is reported, for example, that Otto Warburg was occasionally invited to dinner at the Einstein family home[88] and some of their correspondence from later periods has also survived.[89]

5.2 Influences on Otto Warburg

How is all this related to photosynthesis research? First, as was demonstrated earlier, Otto Warburg explicitly adopted the type of questions introduced and

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pp. 352–353: "Mit Warburg, der in Brüssel war, habe ich auch eine Sache. Er hat unrichtig bewiesen, dass es eine photochemische Reizschwelle geben müsse. Bei dieser Gelegenheit fand ich einen interessanten thermodynamischen Beweis für das photochemische Aequivalentgesetz, das Warburg verifizieren will. 1 Molekül wird von ν-Strahlung zersetzt unter Absorption der Energiemenge hν."

[84] See Einstein (1912*a*) and Einstein (1912*b*). The editorial note to the reprint of the paper in Klein et al. (1995) gives an account of the dispute (and later collaborative work) between Einstein and Emil Warburg on the subject.

[85] See Warburg (1917) and Warburg (1924). Emil Warburg also published extensively on the general energetics of the photochemical reactions of gases: nine articles, all of them entitled, "On the energy conversion in photochemical reactions in gases", appeared between 1911 and 1919 in the periodical of the Berlin Academy of Sciences ("Über den Energieumsatz bei photochemischen Reaktionen in Gasen", published in the Sitzungsberichte der königlich-preussischen Akademie der Wissenschaften).

[86] In fact, Emil Warburg had been one of the members of the Berlin Academy of Sciences who had been behind Einstein's appointment; see, e.g., Goenner & Castagnetti (2004) and Goenner (2005) for details of this particular episode.

[87] See, on this point, Einstein's letter to Otto Warburg, urging him to return to Berlin from the war in July 1918, in which Einstein wrote: "Sie wundern sich gewiss, von mir einen Brief zu bekommen, weil wir bis jetzt nur umeinander herum gegangen sind, ohne einander eigentlich kennen zu lernen." Schulmann et al. (1998), No. 491.

[88] The outcome of one of these occasions was the employment of Hans Krebs in Warburg's laboratories in 1926, as Krebs himself reported. He described the situation as follows: "It so happened that my colleague at the third Medical University Hospital in Berlin and close personal friend Bruno Mendel, three years my senior, was also a friend of Albert Einstein, and at some time in 1925 Einstein invited Mendel and his wife Hertha to a dinner party at his apartment. Another guest was Otto Warburg and at the dinner table he was placed next to Hertha Mendel." Quoted in Werner (1991), p. 137, doc. 45.

[89] See, e.g., the information provided by the project "Einstein Archives Online" on their web site: http://www.alberteinstein.info; last accessed 11 July 2009.

defined by his father in the latter's work on photochemistry. In his article of 1922, by searching for the energy balance and the photochemical efficiency of photosynthesis, Otto Warburg was, in effect, carrying out his research along the same lines as his father. In 1923, in addition to trying to find the quantum requirement of the process, Otto Warburg investigated the influence of the wavelength of light on photosynthetic efficiency. All this work was intimately linked to Einstein's Law of Photochemical Equivalence, which Otto Warburg accepted unquestioningly. And, as was discussed earlier, Otto Warburg applied some of his father's photochemical concepts – the "photolyte" and the notion of "photochemical efficiency" – to his own work.

Furthermore, thanks to his father's position, not only was Otto Warburg able to use the excellent optical instruments at the PTR, he also received a skilful introduction to photochemical experimentation by the experts in the field as well as practical support whenever it was needed. If one takes a closer look at Otto Warburg's biography, there were three periods during which he worked in his father's radiation laboratory: in 1905/06 while studying in Berlin; in 1914 before starting at the KWI for Biology; and, again, in 1918, after having returned from active service in the First World War. It seems that whenever he was in Berlin, Otto Warburg took the opportunity to use the PTR's sophisticated instrumentation. The experiments Otto Warburg carried out at the PTR, starting from 1918, are documented in the PTR's yearly reports, and in his papers Otto Warburg duly gives credit to the support he received from the PTR (he even expresses gratitude to the staff for having carried out measurements on his behalf).[90] It would be natural to assume, therefore, that Otto Warburg simply observed his father's activities and then transferred them to a different – namely, a biological – field of inquiry. However, the actual circumstances are more complicated (and more interesting) than that. If one examines the PTR's annual public activity report for 1911, that is, for the first year of Emil Warburg's research project on the energetics of photochemical reactions, one finds the following programmatic description of this project's goals:

> An important class of photochemical reactions to which belongs, among others, the [photosynthetic] assimilation process in green plants, proceeds with the uptake of energy, which is then retrieved from the absorbed radiation and forms a certain fraction of it. We have taken up the task to measure this fraction, the photochemical yield, for a number of cases.[91]

Thus, as early as the first outline of his photochemical research programme, Emil Warburg explicitly mentions photosynthesis as being one of the principal classes of reactions he wanted to study from the point of view of energetics.[92] Emil Warburg's focus of interest was photochemical efficiency: he took it for granted that only a fraction of the light energy absorbed by a molecule was used for the subsequent chemical reactions, and he wanted to find out what this fraction was in

[90] See, e.g., Warburg (1919), pp. 235 and 255.

[91] Report of the PTR for the year 1911 (Zeitschrift für Instrumentenkunde 22), p. 131.

[92] This view of photosynthesis, as an ideal case study for the laws of photochemistry, was shared by many of Warburg's physicist colleagues; see, e.g., Weigert (1911), for the extensive treatment of photosynthesis in Fritz Weigert's monograph on the chemical effects of light.

particular cases. A second indication that the subject of photosynthesis engrossed Emil Warburg from early on can be found in one of the few surviving letters to his son Otto, dated 9 December 1912:

> [...] Today, I have read in a paper by [Fritz] Weigert* – which, by the way, otherwise contains little of interest – that an Englishman, Brown & Escombe (Int. Trans. Roy. Soc. 183 B 223, 1900; Proc. Soc. 76 B, 1905; Nature, March 1905) has carried out very similar experiments to the ones that we are intending to do. Having scanned Weigert's account, I understand that the process is apparently very complicated, that in particular [photosynthetic] assimilation is largely (1:12) independent of light intensity; this is explained by the fact that the rate of assimilation is determined by CO_2 diffusion.
>
> *[Footnote:] *ZS für wissenschaftliche Photographie, Photophysik & Photochemie.* Vol. 11, issue 2, p. 381.[93]

The letter is particularly interesting as it refers to experiments that father and son were intending to carry out together as early as 1912. Scanning the bibliographic references, one can trace the very article written by Brown and Escombe that Warburg later quoted in his paper as having inspired him to use rotating sectors, the results of which he dismissed as being based on methodically flawed experimentation.[94] Thus, although Otto Warburg only published his first article on photosynthesis in 1919, he had been planning experiments on the theme with his father as early as 1912. One could also speculate that they might have discussed the theme at an even earlier date, perhaps around the time when Emil Warburg mentioned in his PTR report of 1911 that photosynthesis was one of the reactions that were of particular interest to him. Yet, Otto Warburg was then deeply involved in the study of respiration, and the planned experiments had to wait another two years, until Otto Warburg came to Berlin in 1914 and spent some time at the PTR before moving into his new laboratory at the KWI for Biology.

As no laboratory documentation, neither in Otto Warburg's personal estate nor in the PTR's archives, has survived from this period, it is impossible to tell how far research on the theme had advanced when Otto Warburg enlisted. However, neither Emil Warburg nor Carl Correns hesitated to mention Otto Warburg's photosynthesis experiments as an argument for calling him back to Berlin; and even though they might have exaggerated how far the experiments had progressed, it seems unlikely, in view of their dependence on the benevolence of the Ministry of Education, that they would have used this argument had there been no foundation to it. In any event, as can be taken from Correns's report on the activities of the KWI for Biology from 1 April 1918 to 30 March 1919, photosynthesis was the first theme that Otto Warburg took up after returning from the battlefields:

[93] The original letter is preserved in the Archive of the Berlin-Brandenburg Academy of Sciences and Humanities (Archive of the BBAW) in Otto Warburg's estate, at shelf mark NL Warburg 999. A transcription of the German original can be found in Werner (1991), p. 77; doc. 23.

[94] The references cited by Emil Warburg are Brown & Escombe (1900), Brown & Escombe (1905), Brown (1905) and Weigert (1912).The letter was presumably written shortly after Otto Warburg's habilitation in December 1912, since in the rest of the text Emil Warburg writes of the high attendance figures at Otto Warburg's first lecture as newly promoted *Privatdozent*.

> Warburg, the head of department, was already back at work in October 1918, but was only able to use his rooms again at the end of the year. In addition to the repairing and renovating of his premises, he was engaged in studies concerning the assimilation of carbonic acid in green cells, in particular attempting to separate the assimilation process from the cell structure and studying the influence of assimilation in living cells. Besides two laboratory technicians, Professor Warburg collaborated with a student, Miss von Ranke.[95]

According to the 1919/20 report of the KWI, photosynthesis was also Warburg's main research theme in the following year: "[In Warburg's division] work has been done on the assimilation of carbonic acid and nitric acid in the green cells of plants, partly in collaboration with Miss Alexandra [von] Ranke and Mr Erwin Negelein."[96] Thus, on closer investigation, one finds evidence that Otto Warburg, together with his father, was interested in photosynthesis as early as 1912; that by 1914, he had definitely turned to the subject matter, and come back to it in 1918, publishing his preliminary conclusions in the years 1919 through 1925. The war only interrupted a project, which in 1914 was already well under way and which certainly was influenced – and promoted – by Emil Warburg's research aims and the PTR's facilities.

Yet, in his first substantial publications of 1919/20, Otto Warburg made no mention of the reaction's efficiency or its quantum requirement but instead presented a comprehensive investigation of the chemical mechanism of photosynthesis. Although he made use of his father's concepts in these first papers, he only turned to energetics in 1922: in other words the latter did not have priority. It seems that Otto Warburg's interest in photosynthesis was not, after all, exclusively influenced by his father. Rather, one finds a far more convincing source of motivation, not only for the theme in general but also for its specific treatment, if one takes a closer look at Otto Warburg's research up until 1914, in particular his studies in cell respiration.

6 Studies in Cell Respiration

Otto Warburg's research into cell respiration – or biological oxidation, which is the more general term – has been the subject of a number of excellent studies, to which I have referred for the following account.[97] It was for this field of research

[95] Translated from Werner (1991), p. 128. During the war, the rooms of Warburg's laboratory had been used to conduct experiments, headed by Fritz Haber, in poisonous gases; consequently, to make the rooms amenable to the study of living organisms again, all the toxic residuals from the floors and the benches had to be removed.

[96] Cited in Werner (1991), p. 146.

[97] For Otto Warburg's early concept of *Atmungsferment*, see, in particular, the thorough study by Kohler (1973*a*); see also Höxtermann (2007) for an explication of Otto Warburg's understanding of biocatalysis. The subject matter is also treated in, e.g., Höxtermann (2001) (pp. 265–268) and Werner (1991) (pp. 64–69; 113–118). Werner (1996), a careful edition of Otto Warburg's correspondence with people such as Jacques Loeb and Leonor Michaelis, also deals with the problems of a theory of respiration; a comprehensive introduction provides rich historical background. Werner (1997) analyses the controversy that arose between Otto Warburg and Heinrich Wieland on the theory of biological oxidation. More specific references can be found in all these publications.

that Warburg was most famous and for which he received the Nobel Prize for Medicine or Physiology in 1931. However, this section focuses on the early years of Warburg's research, that is, the period 1908 to 1914, which means that Warburg's final success, his concept of *Atmungsferment*, which was developed in the 1920s, has been omitted from the discussion.[98]

Cell respiration was the first theme that the young Warburg chose to study independently, choosing it for both his medical dissertation in 1911 and for his habilitation in 1912. Warburg's work in these years was strongly influenced by Jacques Loeb's "mechanistic conception of life" and the programme to explain life processes in a physico-chemical way.[99] Warburg frequently went to a research institute in Naples, the Zoological Station, where he met the avant-garde of this new research tradition, which included Hans Driesch, Oscar Hertwig, Curt Herbst, Theodor Boveri and Thomas H. Morgan. However, although Warburg received much inspiration from their work on developmental physiology, he quickly set himself his own research goals, which were mainly concerned with the physico-chemical elucidation of energy-producing reactions in general – a subject that was then extremely controversial.[100]

As with photosynthesis, Warburg also completely – and revolutionarily – changed research in respiration by introducing both new techniques and new conceptual approaches.[101] Warburg was the first to study biological oxidations in isolated cells; up to then the test organisms used for this purpose had been mice, rabbits and other animals, which made even approximate compliance with the homogeneity condition extremely difficult. For his first studies, Warburg investigated sea urchin eggs, which were then the main test objects employed in the field of developmental biology. Indeed, it is also very likely that Warburg originally intended to study the problems of embryonic development. In his first publication on the subject, he stated that he would carry out further investigations into the chemical reactions in the early cleavages of the fertilised egg; the rate of oxidation was the first parameter he turned to.[102] In other words, studying the respiratory processes of the fertilised egg was only a sub-goal of the superordinate goal – to understand the underlying chemical mechanisms of early embryonic cell cleavage. However, this sub-goal turned out to be so interesting that Warburg very quickly made it the main focus of his research – although he chose not to change his test object.

As Warburg was convinced that the mechanism of cell respiration was the same in all cells, it did not matter to him from which organism the material was gathered. However, which units were chosen was relevant. Since it is far easier to carry out controlled experiments on simple systems, the preferred choice for quantitative studies are the smallest possible units – as in photosynthesis, the reason being the homogeneity condition. Single cells are far easier to reproduce in comparable states

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[98] I am grateful to Ekkehard Höxtermann, Berlin, for the extensive discussion we had on this theme, which greatly improved this section; in addition, the thesis work done by Rahel Ringger, Bern, on the subject helped me to clarify some points of the story.

[99] Cf. Loeb (1905). For information about Loeb's influence on Warburg's research, see, e.g., Werner (1996), pp. 35–47.

[100] See, e.g., Kohler (1973*a*), Kohler (1973*b*) and Werner (1996).

[101] Cf. Kohler (1973*a*), p. 183.

[102] See Warburg (1908), p. 1.

than entire mice or rabbits.[103] It was also during the course of his studies in cell respiration that Warburg developed his sophisticated technique of manometry by adjusting the then available instrument, the Haldane-Barcroft manometer, to the requirements of his investigations (incidentally, it was his father, who, in 1900, had described the first differential manometer, that is, it was Emil Warburg who had laid the foundation for all the later refinements of the technique by his son).[104] In his manometric experiments, Warburg found new ways of using the reversible inhibition of cell processes by different anaesthetics as a means of investigation; in particular, he used surface-active substances, such as urethanes, and the heavy metal-binding cell poison, hydrogen cyanide. Indeed, it was Warburg who, through his own work, established how these substances were able to inhibit respiration, either through their adsorptive capacities or through a chemical reaction.[105] Thus, a large part of the equipment and substances that Warburg would later so innovatively introduce to photosynthesis research had originally been employed in his earlier studies in cell respiration: the use of single cells as the test object, the technique of manometry and the use of a range of inhibitors, notably hydrogen cyanide and urethanes.

Furthermore, the principal question that Warburg raised in his articles was the same question that he would set himself in his photosynthesis studies: Why are substances, which at room temperature are usually extremely stable, subject to very fast combustion in living cells?[106] Some sort of catalysis, it seemed, had to be involved; but then, how should this catalysis be described? Following Eduard Buchner's discovery of "zymase" (an intracellular enzyme complex) in 1897, two options presented themselves: the decisive factor was either the action of the cell structure (the biologists' opinion) or the action of an enzyme (the chemists' view). Warburg solved this issue by integrating both modes of catalysis into one complex mechanism, as he repeatedly emphasised in his papers as well as in a speech of 1914:

> I hope I have demonstrated to you today that there is no dichotomy here at all: both ferment chemists and biologists are right. The acceleration of energy-producing reactions in cells is a ferment action *and* a structure action; it is not that both ferments *and* structure accelerate, but that *structure accelerates ferment action*."[107]

Through his use of surface-active inhibitors, Warburg was able to establish over the course of the years that internal cell surfaces were usually essential for cell respiration. He proposed that a "ferment" was involved, which would accelerate the oxidation processes, and that the action of this ferment itself was greatly accelerated when it was attached to the structural elements of the cell. This

[103] See, e.g., Warburg (1914), p. 320, where he discusses the advantage of working with cells rather than with tissue.

[104] See Warburg (1900), p. 712.

[105] On the history of using anaesthetics in respiration studies in general and on the discussion centred around Warburg's use of them in particular, see Werner (1996), pp. 87–95, and Werner (1997), pp. 183–190.

[106] See, e.g., Warburg (1914), p. 314.

[107] Warburg in 1914; translation taken from Kohler (1973*a*), p. 190.

concept of the process rested on Warburg's personal notion of what a ferment was: while Buchner and others "considered enzymes to be definite proteins with specific catalytic properties [...] Warburg renewed the colloid chemists' ideas of surface activity as an attractive alternative". This is why Warburg never gave up the somewhat old-fashioned term "ferment", which implied that it was not a single protein that promoted a certain process but the cell as a whole.[108] The fact that respiration was so sensitive to the influence of hydrogen cyanide, which readily binds with heavy metals, was taken by him to be evidence that heavy metals of one kind or another were the active part of this ferment. In 1914, Warburg finally came to the conclusion that this heavy metal was the cell's iron, which acted catalytically to promote oxidation by being reduced from its ferric (Fe III) to its ferrous (Fe II) state.

Warburg was very cautious about the possible presence of intermediates in the process, and for a long time did not even speculate about the elusive substance, X, which was the first substance to be oxidised. However, in his review article of 1914, Warburg suggested that the relevant mechanism included the "oxidation of lipoids in the presence of iron salt".[109] Warburg concluded this from his experiments with lecithin, which he seems to have taken to be representative of the whole group of lipoids. The assumption seemed coherent with the general body of knowledge at that time, since it was known that the internal cell structures that Warburg considered so important were, in large part, made up of lipoids. Thus, in Warburg's model of 1914, lipoids were part of the structure on to which the iron ferment was adsorbed as well as the actual substances on to which the oxygen was transferred through the action of the iron ferment. The emerging model is graphically reconstructed in figure II.13.

Warburg was aware that this was not the final word on the issue. He was, for example, silent on the actual sequence of the initial reaction steps – how the oxygen was brought into contact with the iron ferment (was it first bound to surfaces as well?) and whether there were other components of the structure, in addition to lipoids, that were significant for the full process of cell respiration. But even so, as early as 1914, Warburg was able to present an impressively detailed account of cell respiration, based on rich empirical evidence for the postulated causally relevant relationships.

The similarities between this model and Warburg's photosynthesis model of 1919/20 are obvious. Warburg's early research in cell respiration enabled him to formulate his later views on photosynthesis. The processes were framed in exactly the same way – heavy-metal-catalysed reactions, which occurred on internal cell surfaces – and Warburg used exactly the same techniques to provide evidence for this general view of events. And that these similarities were intentional can also be proved. In his 1927 book, *The Catalytic Action of the Living Substance* (*Katalytische Wirkungen der lebenden Substanz*), a collection of selected papers

[108] Quoted in Höxtermann (2007). For the complicated development of the concepts of "enzyme" and "ferment", see, e.g., Fruton (1972), Kohler (1973*b*) and Teich (1981).

[109] Warburg (1914), p. 335. Original German expression: "Oxydation von Lipoiden bei Gegenwart von Eisensalz."

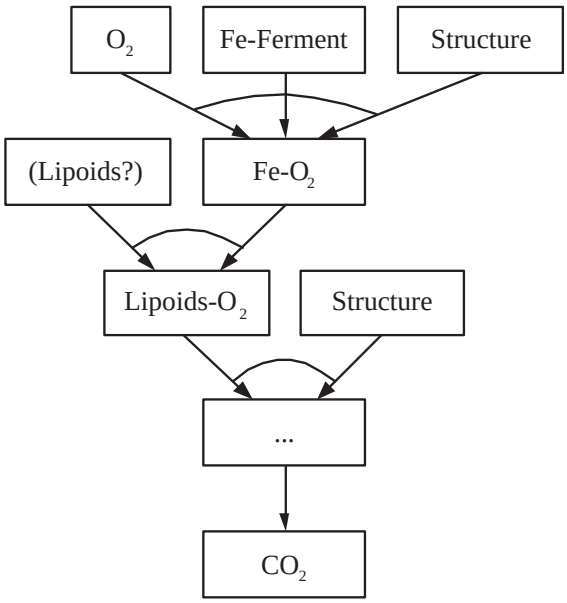

Figure II.13: Warburg's 1914 model of cell respiration.

by Warburg on cell respiration and photosynthesis up to that time, he wrote the following:

> Heavy metal catalysis [such as respiration] is also the Blackman reaction, which is part of the process of photosynthesis, [...]. [...] If I may add that these reactions are also surface reactions, one realises that the most important catalytic actions of the living substance are based on the same principle. The kind of metal and the type of bonding may vary, but the principle remains the same.[110]

Warburg was convinced that fundamental principles govern the chemical reactions in all organisms, from bacteria to human beings, which was why he experimented with cells as specific as sea urchin eggs and still did not hesitate to generalise his results to the entire living world. Warburg also believed that the life processes in plants and animals were fundamentally the same and, therefore, should not be investigated in isolation from each other. In her diaries, Warburg's sister, Lotte, writes that, in 1926, Warburg commented on Correns, who was irritated by the fact that the field of animal physiology was expanding, thereby displacing plant physiology, his own field of inquiry. Warburg, his sister wrote, considered this point of view ridiculous and narrow-minded: "What, however, is the difference? This all belongs together, after all."[111]

Of course, notwithstanding the similarities in Warburg's accounts of respiration and photosynthesis, there were obvious differences; in photosynthesis, for example, Warburg had to accommodate the role of chlorophyll. But the general picture

[110] Warburg (1927), p. 12.
[111] See Rüskamp (1989), p. 252; also quoted in Werner (1991), p. 143.

is undeniably the same: the same techniques, the same conclusions, the same conceptualisation of reaction steps. Given Warburg's interest in energy-producing reactions (not only in respiration but also in fermentation), his expertise in manometrically measuring gas exchange as an indicator of reaction rates, and the fact that he had been highly successful in elucidating by this means the mechanism of cell respiration, it required only a small step to apply this sophisticated package of methods, instrumentation and concepts to other related fields of inquiry. Warburg was convinced that the energy-producing reactions in all respiring organisms were essentially the same; thus, why not examine the second large class of energy-producing reactions, namely photosynthesis, and see whether similar principles held there? Warburg's shift to studying photosynthesis seems, from this perspective, to be an example of research opportunism in action *par excellence*. It also explains why, in his studies in photosynthesis, Warburg at first used the methods taken directly from his earlier research, and why he only seriously became involved in photosynthesis after 1918, when he could no longer travel to Naples to obtain sea urchin eggs for his studies in respiration. Clearly, Warburg had decided that it was also worth his while to study the subject matter from the point of view of energetics, which had also interested his father. It is interesting to note, however, that although Warburg continued to publish occasionally on photosynthesis for a couple of years after his 1923 paper, he would never again devote so much time and energy to it .

To sum up: there is much evidence to support the assumption that Warburg contributed to photosynthesis research only opportunistically, perhaps in order to make good use of the time during which he could not work on his studies in respiration because of a poor supply of his test object – sea urchin eggs. Warburg then found that he could, in actual fact, make a contribution to the subject; yet after 1925 he resumed his studies in cell respiration and also turned to the study of cancer. The year 1925 would have marked the end of Warburg's work on photosynthesis, had it not been for the controversy that arose, after 1945, on the maximum quantum requirement, which forced Warburg to focus his attention on these questions again.

7 Comparison with the Chlorophyll-Complex Model

To round off the picture, I shall now briefly discuss how Warburg's model relates to the lines of research that were followed in Chapter I. In figure II.14, Warburg's proposal has been juxtaposed with the chlorophyll-complex model of Willstätter and Stoll, which was published in 1918 and, as was mentioned earlier, was considered to be the most satisfactory photosynthesis model at hand.[112] The similarities are striking. At first glance, the only major differences are the conception of the primary action of light and Warburg's addition of a surface-dependent iron ferment, which produces the purported reactive carbon derivative and which only afterwards binds to chlorophyll. In fact, Warburg seems to have used the suggestion made by Willstätter and Stoll as his starting point, which he then extended and modified on the basis of his experimental data (although he never mentioned this procedure in his papers).

[112] The model was published in Willstätter & Stoll (1918).

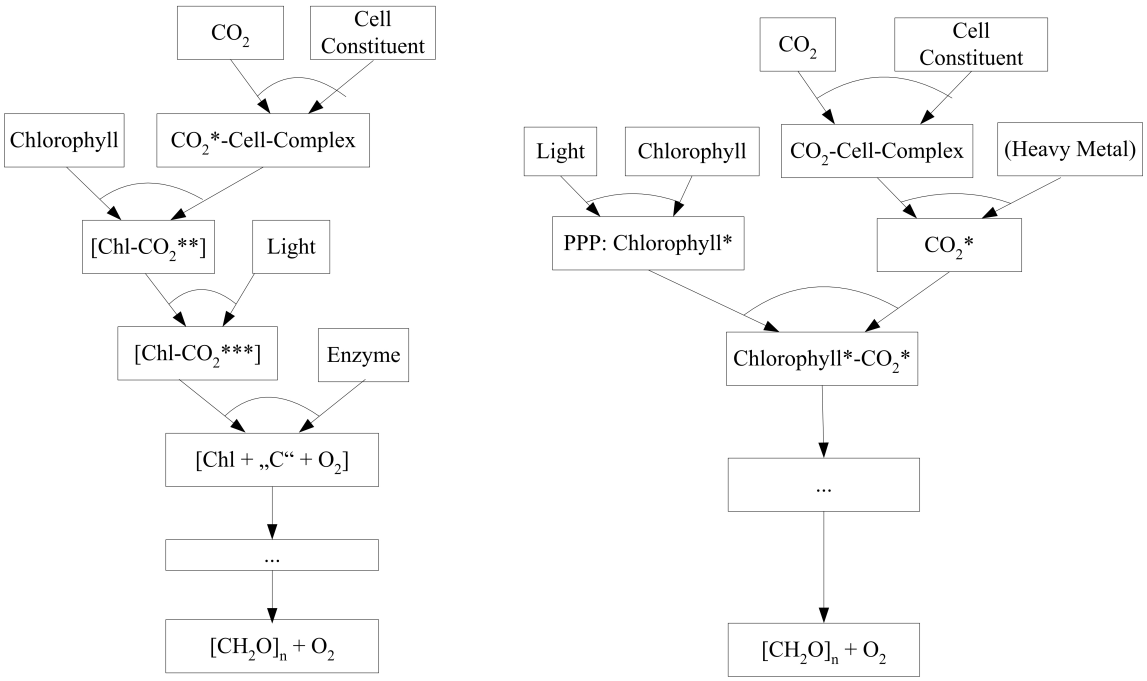

Figure II.14: A comparison between the photosynthesis models of, left, Willstätter & Stoll (1918) and, right, Warburg (1919, 1920).

Warburg had evidence, for example, that a surface-dependent, thermochemical reaction occurred that was sensitive to hydrogen cyanide (which implied that the the reaction in question most probably involved the action of heavy metals). Kinetic data also suggested that carbon dioxide (respectively carbonic acid) was involved in this reaction as well as an additional compound of unknown nature. Warburg identified this compound as part of the cell's constituents, with which the carbonic acid reacted; and he further assumed that heavy metals, which were somehow embedded into the cell surfaces, would activate carbonic acid in this complex binding to one of its derivatives. The latter was simply an extension of Willstätter and Stoll's model hypothesis through the addition of an extra cofactor to the carbonic acid-cell complex module.

Willstätter had also thought that chlorophyll became part of this complex, upon which the action of light would then activate the carbonic acid derivative again, before the actual reduction took place through the action of an enzyme. Warburg would later identify this complex as the "photolyte": the compound that was the subject of the actual photolysis, that is, light splitting.[113] Warburg again modelled the processes in a similar, but not identical, way. According to his experiments, the primary photochemical process resulted in the production of a strong reducing agent, which he thought was activated by chlorophyll. Thus, the light would only act on the chlorophyll, which then in its activated form would induce the reduction

[113] See Höxtermann (2007) for a comment on Warburg's concept of the "photolyte", which is closely connected to his understanding of biocatalysis and which he developed over time.

of the carbonic acid derivative. This made the action of an additional enzyme superfluous. The remaining steps to the carbohydrate stage then were the same, since Warburg agreed with Willstätter and Stoll that possibly formic acid and peroxides of some kind were involved.

Thus, although Warburg gathered his data by carrying out completely different experiments, using other methods and a new test organism, he modelled his findings very much in line with the standard assumptions of the time – combined with elements that he had taken from his earlier modelling of respiration, such as the involvement of a heavy metal as a catalysing agent. This explains why Warburg's model was only rarely received as an original contribution to photosynthesis research – even though, as far as their experimental foundation was concerned, Warburg's papers were highly innovative. The fact that most of the causally relevant factors postulated in the chlorophyll-complex model of Willstätter & Stoll were also inferred by Warburg, albeit from totally different sets of data, was rather taken to corroborate strongly the earlier suggestion, which was well on its way to becoming the new "standard model".

8 Otto Warburg's Goals and Building Blocks

As demonstrated in this chapter, the work that Warburg carried out in the field of photosynthesis between 1919 and 1925 was a direct consequence of his research goals and methods of the years 1908 to 1914. Early in his career he focused on the energy-producing reactions of metabolism, in particular those reactions that could be investigated using manometric techniques – that is, gas exchange processes. After achieving considerable success in the fields of respiration and fermentation, the next obvious challenge – given Warburg's general conviction, much the same as Jacques Loeb's, that all fundamental life processes were based on similar principles – was the investigation of the curious energy-producing mechanisms of plants. The second package of research goals that fundamentally influenced Warburg's work in these years were the studies of his father Emil Warburg, which were part of a general attempt being made by physicists working in Berlin at the time to explain natural phenomena in terms of quantum laws. Emil Warburg chose to explore photochemistry in this respect; and photosynthesis, a natural example of a photochemical mechanism, seemed the ideal subject to study. It would be entirely reasonable to assume that, as a physicist, Emil Warburg felt himself unable to handle working with living organisms, so that he tried to convince his son to collaborate with him. In exchange, Emil Warburg could offer Otto the use of the PTR's sophisticated photophysical instrumentation as well as the help of collaborators, who could introduce him to these techniques.

Otto Warburg's unconventional approach to the study of photosynthesis – as well as his use of very specific instruments – can, with certainty, be attributed to the work that he carried out on cell respiration while in his late twenties. His later practical and conceptual work was very much organised according to the principles that he had developed before the start of the First World War. He continued to use manometry as a measuring technique and single cells as test objects; and he adhered to the assumption that surface-dependent heavy metal catalysis was the fundamental principle of the energy-producing reactions of respiration,

fermentation and photosynthesis. And, like his father, Warburg believed that the concept of photolysis was the essential component of photochemical reactions, which also fitted Warburg's own notion of fermentative action. Finally, Warburg used the Willstätter-Stoll model of photosynthesis, with its complex of chlorophyll and a carbon dioxide derivative, as a starting point for his own modelling of the process, to which Warburg then introduced other cofactors and intermediate steps to accommodate his new empirical findings.

Thus, Warburg's research pathway not only exemplifies the principle of research opportunism, which was what brought him to study photosynthesis in the first place; it also demonstrates the building block strategy that was introduced in Chapter I. While researching into photosynthesis, Warburg had a superordinate goal, and to achieve this goal he used a number of techniques, instruments and concepts that he had acquired from his own experience as well as from other scientists. These included: (1) the standard body of knowledge of the time; (2) Warburg's own achievements in his earlier studies, albeit not in the same field of research; and (3) the highly successful concepts of his father, which the latter had employed to explain related phenomena.

Warburg's research pathway can be explained by following his various goals, sub-goals – and incidental goals. His original line of interest – to explain physiological processes in terms of physico-chemical concepts and laws – was fostered on his visits to Naples, where Warburg came into contact with the most eminent developmental biologists of the time, notably Loeb and his colleagues, who were engaged in setting up the discipline of general physiology. Warburg's first studies in the oxidation processes in sea urchin eggs were obviously inspired by the goal of contributing to this line of research in developmental biology, which was then at the forefront of biological sciences.

However, over time, Warburg turned from his original sub-goal of trying to clarify the oxidative processes in sea urchin eggs and the role of these processes in their development to the goal of elucidating cell respiration, which then led him to set himself a new superordinate goal, namely, to explain all the energy-producing reactions of metabolism. Having successfully looked at respiration and fermentation, Warburg, in a typically research-opportunistic manner, turned to photosynthesis. Earlier in this chapter, I presented the arguments for assuming that the idea of studying photosynthesis had been on Warburg's mind from as early as 1912. At the same time, I also do not believe that it was purely coincidental that Warburg only seriously and exclusively turned to this subject in 1918, after having returned from the war. To be able to resume his studies in cell respiration – which he was presumably keen to do in view of the fact that by the time he had to discontinue his studies in 1914, his work was looking very promising – he would have had to travel to Naples, because at the time his research depended on the availability of sea urchin eggs; yet in the early years of the Weimar Republic, money was not available for journeys of that kind. Thus, Warburg was forced to become involved with research questions that could be carried out using more mundane test organisms – such as unicellular freshwater algae.

9 Warburg's Impact on Photosynthesis Research

Even though Warburg may have come to study photosynthesis partly by chance, in some ways he was exactly what the field needed, providing much-needed new momentum. Whereas in Chapter I the different collectives working in photosynthesis research were presented as being widely distinct, Warburg was one of the few people at the time who was able to combine the numerous research goals and methods to form a new approach. He still pursued the goal of the chemists – to find the photosynthetic mechanism – yet, in order to do so, Warburg not only picked up interests and methods from cutting-edge quantum physics and photochemistry, he also availed himself of new biological techniques and employed the methods that he had developed for his physiological work. And, by interpreting his measurements of photosynthesis rates in terms of reaction mechanisms (a technique that later became very popular, especially in enzyme studies, but was still at this time a novelty), Warburg made good use of the progress made in the basic concepts of chemistry.

The extent to which Warburg influenced the field of photosynthesis research cannot be overestimated. All the research groups working on this theme adopted manometric methods and by the 1930s the fruitless search for chemical intermediates had largely been dropped; kinetic studies using gas exchange measurements became the standard approach. Manometric studies provided information on the chemical and physical details of the photosynthetic reaction, which up to then had not been obtainable. Yet these changes did not take place as quickly as one might have expected. The German plant physiologist André Pirson wrote in an autobiographical account that, even in the 1930s, the application of manometric techniques was mostly unheard of in general institutes of botany, and even a decade after Warburg's first papers had been published, the use of unicellular algae as test organisms remained unfamiliar to most botanists.[114] One of the reasons may have been that Warburg had developed his seminal contributions not at a university but at one of the new Kaiser Wilhelm Institutes. Although these institutes were marvellously equipped and had access to high-quality instrumentation (as well as instrument builders), they were also somewhat isolated from mainstream science. Thus, Warburg's (and Willstätter's) findings and particularly their techniques only slowly found their way into university curricula. Consequently, since students of botany were not usually exposed to these cutting-edge findings and methods but had to make do with more traditional approaches, these new fields did not attract many young and promising researchers. Most biological curricula were dominated by animal and plant morphology, while plant physiology, if it was taught at all, continued to be predominantly centred on questions of, for example, cell permeability, movement in plants and, perhaps, respiration. Photosynthesis remained a side issue, a situation that would not change until after 1945. However, the 1930s saw a sharp increase in new and fascinating revelations, which are the subject of the next chapter.

[114] See Pirson (1994).

Chapter III

STRUGGLING WITH THE STANDARD MODEL (1930–41)

1 Introduction

In the early 1930s, photosynthesis research was still far from being the popular research 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. Only a small number of scientists studied the subject over a long period of time, and those who did were closely interrelated by friendship, personal collaborations or teacher-student relationships. It is interesting to note that most of the main research in photosynthesis research was undertaken in only a small number of places: in Berlin (Germany), in Pasadena and Chicago (United States) and in Cambridge (United Kingdom).[1] Thus, even though there were few central actors, they came mostly in clusters, and, of course, there continued to be a group of opportunistic contributors, who at times provided seminal contributions to research in the field.

However, it was in this decade that, for some scientists, photosynthesis started to become a theme worth spending a lifetime of research on, among them 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.

If one were to single out a common feature of the experiments carried out in this period, it would be the application of the technique of manometry to the studies on the metabolism of the unicellular green alga *Chlorella*. Many of those scientists who would later become world experts in photosynthesis spent an extended period of research at Otto 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 not 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 by a research stay at Emerson's laboratory. Franck never worked directly with Warburg, but they nevertheless knew each other well from the time Franck spent in Berlin (Franck 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.

[1] The universities of Berkeley and Urbana–Champaign in the US would soon become equally important centres of photosynthesis research.

As the setting's actors were so closely intertwined, it was difficult to organise the material of this chapter, which can, after all, only be presented one section after the other. I was, therefore, obliged to introduce a number of breaks into the structure that would otherwise not have existed. I start with a discussion of the establishment of a new standard model, 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, for the first time, of fluorescence as a way of investigating the photosynthesis mechanism. The contributions Franck made to extending the Willstätter–Stoll–Warburg model of photosynthesis helped make the latter the "received view" of photosynthesis in this decade. The remainder of the chapter then presents the various challenges to the standard model that arose during this decade, and discusses how the different actors reacted to them.

2 Fluorescence and the Standard Model in the early 1930s

2.1 The Kautsky Effect

The most favoured photosynthesis model in this period remained the one that had been developed by Willstätter and Stoll and then refined by Warburg. Then, beginning in 1931, the German chemist Hans Kautsky began to approach the problem from a totally different angle: he started working on the fluorescence of chlorophyll solutions.[2] This new procedure had far-reaching consequences on further developments in the field.

Around 1930, Kautsky initiated a project to research the energy transformation processes on boundary layers (*Grenzflächen*) in Heidelberg (Germany). This resulted in a series of substantial papers, co-authored by different members of Kautsky's research group, which also covered the first quantitative and systematic study of the fluorescence of chlorophyll. 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]

Although Kautsky and his group were not the first chemists to examine chlorophyll and its fluorescence, they were the first to interpret systematically the resulting data in terms of an underlying mechanism. This approach was based on the observation that, although chlorophyll solutions usually exhibited an intensive and beautiful 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

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

[3] See Kautsky & Hirsch (1931) and Kautsky, Hirsch & Davidshöfer (1932) for the first reports of these phenomena, while Govindjee (1995) provides a historical review of the "Kautsky effect" and Govindjee (2004*a*) covers the phenomenon of chlorophyll *a* fluorescence from both a historical and a systematic viewpoint.

served as a convenient indicator of the efficiency of the suspension's photosynthetic activity.

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, 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 increasingly 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] Kautsky et al. (1932) suggested that the molecule to which the chlorophyll transferred the absorbed light energy was, in both situations described above, molecular oxygen. Based on this assumption, Kautsky and his group developed a detailed theory of "sensitised photooxidation". They surmised that in this process an activated, metastable state of molecular oxygen was involved, which 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. Since Franck would become one of the major players in photosynthesis research, his entry into the field deserves a short biographical detour.

2.2 James Franck and Photosynthesis

James Franck (see fig. reffranck, p. 113) is usually remembered for his seminal contributions to physics *sensu stricto* rather than for his work in photosynthesis.[6]

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

[5] See, e.g., Kautsky et al. (1932), Kautsky, de Bruijn, Neuwirth & Baumeister (1933) and Kautsky, Hirsch & Davidshöfer (1935). However, this suggestion was contested by Hans Gaffron, who was then working in Otto Warburg's laboratory, and held a different concept of photooxidation. Gaffron had studied the photosensitised oxidation of thiourea by, among other substances, chlorophyll, the mechanism of which he interpreted in a totally different way. In contrast to Kautsky's assumption, Gaffron argued that photosynthesis started without oxygen. He believed that if no oxygen were present, fermentation, which was harmful to photosynthesis, would occur; he thus concurred with Willstätter and Stoll's finding – that for photosynthesis to take place small amounts of oxygen were necessary. See Gaffron (1935) for the pertinent publication. It was presumably through his follow-up of these discussions that James Franck first became acquainted with Gaffron's work.

[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

Franck started his academic career as a doctoral student of Emil Warburg in Berlin, where Franck 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. The celebrated paper Franck & Hertz (1914) that arose from their collaborative work provided convincing evidence that energy quanta, as postulated by Max Planck in 1900, did exist, and earned Franck and Hertz the 1925 Nobel Prize in Physics. 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 extremely 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.[7] 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 professorship at Göttingen in protest. This courageous step caused an enormous stir, nationally and internationally, among scientists, politicians and the wider public.[8] 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 (US), 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 take up in 1935. It was during these

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consequences, notably his scientific migration to photosynthesis research. See also, more recently, the extensive biography by Lemmerich (2007), which also includes much valuable background information and quotes from a broad range of original, hitherto neglected documents.

[7] 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).

[8] 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.

Figure III.1: James Franck (1882–1964).

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.[9] 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 the fluorescence of green leaves with Hilda Levi, a young molecular spectroscopist.[10] In addition, Franck collaborated again with Rabinowitch, who had in the meantime also emigrated to Copenhagen, on the solution effects in photochemical processes.[11] In an interview with Levi, Beyerchen learned that Franck had, at first, not been at all interested in photosynthesis as such; he became involved only because chlorophyll made good fluorescing solutions, which could be used to study the underlying energy exchange processes of photosynthesis. At the time these processes, in particular the mechanism of sensitised photooxidation, were the subject of highly controversial debates.[12] However, Franck must have become interested in photosynthesis shortly thereafter, since in the very same issue of the journal *Naturwissenschaften*, in which he published his findings with Levi,

[9] See Beyerchen (1996), pp. 77–79.

[10] See Franck & Levi (1935*b*) and Franck & Levi (1935*a*) for the resulting publications.

[11] See Franck & Rabinowitch (1934), in which they showed how, because of a "cage" of solvent molecules, the quantum yield could be much more than the value of 1 obtained from a chain reaction.

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

Franck also published his first conceptual paper on the photochemical mechanism of photosynthesis.[13] 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 presented his ideas to the English-speaking world for the first time;[14] in the following decades Franck published a series of increasingly sophisticated physico-chemical photosynthesis models, which he developed with a number of co-authors.[15]

In 1938, Franck was invited 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".[16])

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."[17] 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

[13] See Franck (1935*a*).

[14] See Franck (1935*b*).

[15] See, e.g., Franck & Herzfeld (1937), Franck, French & Puck (1941), Weller & Franck (1941), Franck (1945) and Franck (1949). Franck's final attempt to solve the problem was completed shortly before his death: see Franck & Rosenberg (1964).

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

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

> of a living cell depend on its whole history, and thus on more variables than can be reliably controlled.[18]

Only a few of Franck's colleagues 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 intricate 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 the own pet hypotheses.[19]

2.3 The Willstätter–Stoll–Franck Model

Stoll and Willstätter Again

Based on the findings presented by Kautsky and his group, Stoll and later Willstätter made another attempt to solve the problem of how chlorophyll acted in photosynthesis. Confirming the suggestions of Willstätter & Stoll (1918), in 1932 Stoll reported his recent 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.[20] 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, which chlorophyll so strongly attracted. (Chlorophyll was found to be so hygroscopic that it was very hard to isolate the water-free molecule.) Chlorophyll, Stoll surmised, might be able to decompose water under the influence of light, possibly according to the equation:

$$2H_2O \xrightarrow[\text{light}]{} 2H + H_2O_2 \tag{III.1}$$

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 Willstätter & Stoll (1918) that a temperature-dependent process occurred during photosynthesis, which most probably involved

[18] Quoted in Beyerchen (1996), p. 82–83.
[19] Cf., e.g., Franck (1935*b*), p. 433.
[20] Stoll (1932), p. 957.

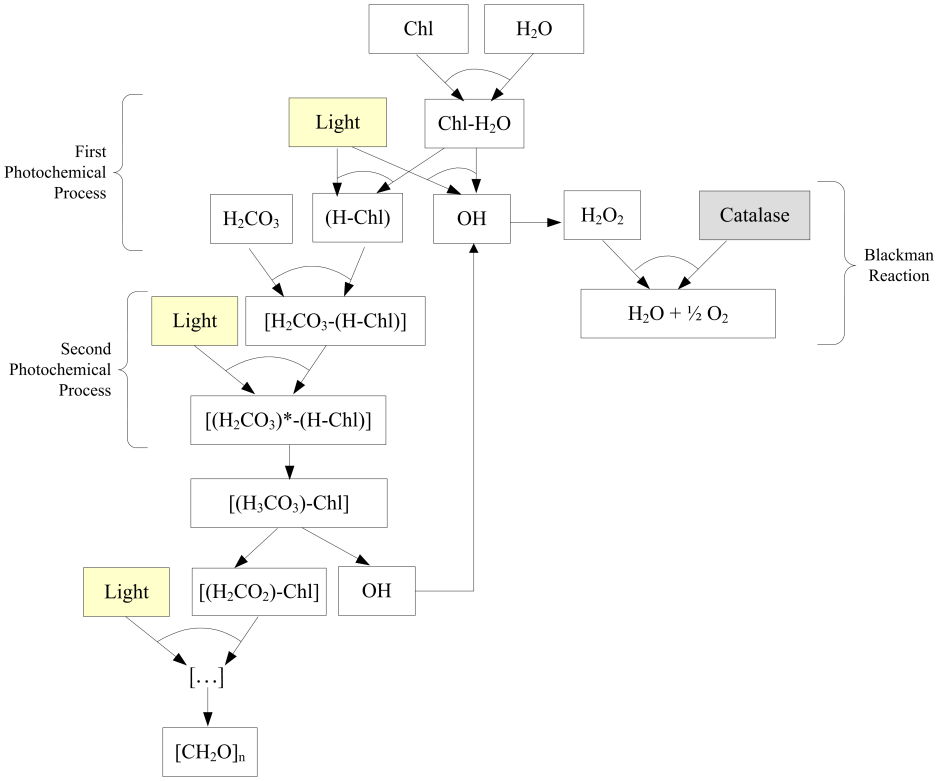

Figure III.2: The extended model of photosynthesis proposed by Stoll (1932).

an enzyme similar to catalase, and which prompted the decomposition of hydrogen peroxide.[21]

Figure III.2 (p. 116) shows this new photosynthesis model in graph form. Water binds to chlorophyll, forming the complex Chl-H_2O. This is decomposed under the influence of light, whereby chlorophyll is hydrogenated to H-Chl. This is the first photochemical process. The simultaneously formed OH radicals would, most probably, combine to hydrogen peroxide. The latter then is immediately 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 is 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 formyl derivative bound to chlorophyll (H_2CO_2-Chl) and a hydroxyl radical

[21] 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 & Uyesugi (1924) was particularly influential in this respect. It was only in Emerson & Green (1937) that the supposed similarity between the Blackman reaction and the reaction between catalase and hydrogen peroxide was contested.

(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, whereupon another hydrogen transfer (and loss of oxygen) would eventually yield a formaldehyde derivative chlorophyll complex (H_2CO-Chl), from which the formaldehyde was released and underwent 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 bonded 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.[22] In this model, reconstructed in graph form in figure III.3 (p. 118), oxygen was, in fact, needed for photosynthesis to take place, namely for the dehydrogenation of chlorophyll: oxygen oxidised chlorophyll (that is, 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 $Mg(H_2CO_3)$ complex (Willstätter also took it for granted that carbonic acid would bind to the chlorophyll's magnesium):

$$\begin{aligned} Chl + O_2 &\longrightarrow O_2H + Chl^{DH} \\ Chl^{DH} + CO_3H_2 &\longrightarrow [CO_3H_2 + H] + Chl^{DDH} \end{aligned} \tag{III.2}$$

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

$$\begin{aligned} Chl^{DDH} + H_2O &\xrightarrow[\text{light}]{} OH + Chl^{DH} \\ Chl^{DH} + [CO_3H_2 + H] &\longrightarrow [CO_3H_2 + 2H] + Chl^{DDH} \end{aligned} \tag{III.3}$$

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, which sufficed for the complete reduction of the carbonic acid molecule. Thereafter (that is, as soon as the magnesium complex was

[22] Willstätter (1933).

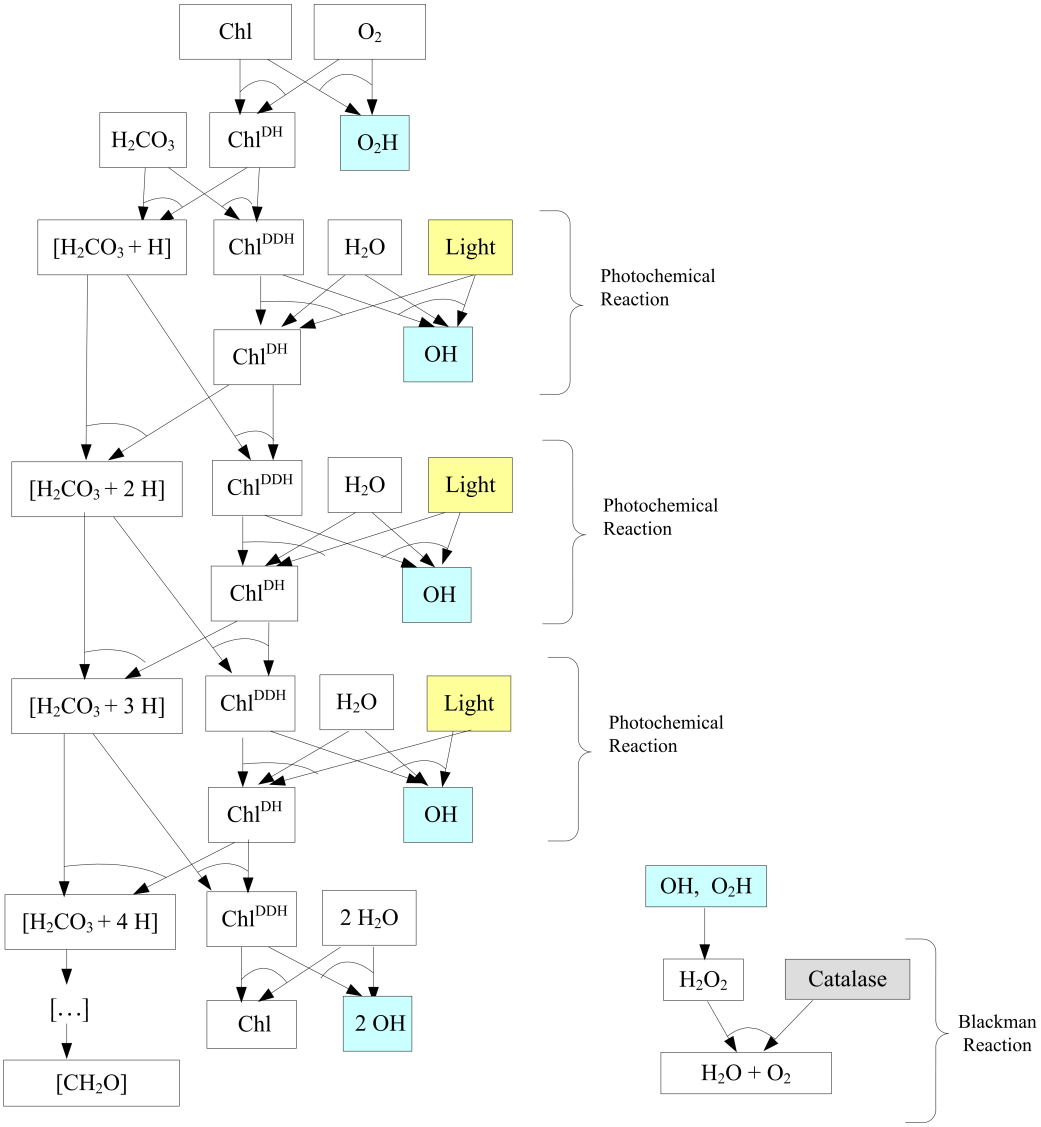

Figure III.3: Willstätter's photosynthesis model (1933).

fully saturated and carbonic acid completely reduced), 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 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. Note, however, that 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 "extended".

Franck Joins the Field

This was the state of affairs at the time that Franck published his first contribution to photosynthesis studies in 1935.[23] He conceded that, in 1918 and then in their contributions of 1932/33, 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.[24]

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 Chapter II, Section 4). Franck was equally dissatisfied with Kautsky's explanation of the course of photosynthetic 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 graph form in figure III.4 (p. 121). In his paper, Franck emphasised that the following conditions had to be met:

> (i) If four 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.

[23] Franck (1935*a*) and Franck (1935*b*).
[24] Franck (1935*b*), p. 433.

Hypotheses about metastable states with a long life time were likewise ruled out because the reactions took place in a condensed system.

(ii) For each photochemical partial reaction the energy of one 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.[25]

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). Franck maintained that this was far too costly, energetically speaking. 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:

$$\mathrm{Chl} \xrightarrow[\text{light}]{} \mathrm{Chl}^{DH} + \mathrm{H} \tag{III.4}$$

(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 quickly used up in the following processes:

$$\begin{aligned} \mathrm{H} + \mathrm{O_2} &\longrightarrow \quad \mathrm{O_2H} \\ \mathrm{O_2H} + \mathrm{O_2H} &\longrightarrow \quad \mathrm{H_2O_2} + \mathrm{O_2} \end{aligned} \tag{III.5}$$

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

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

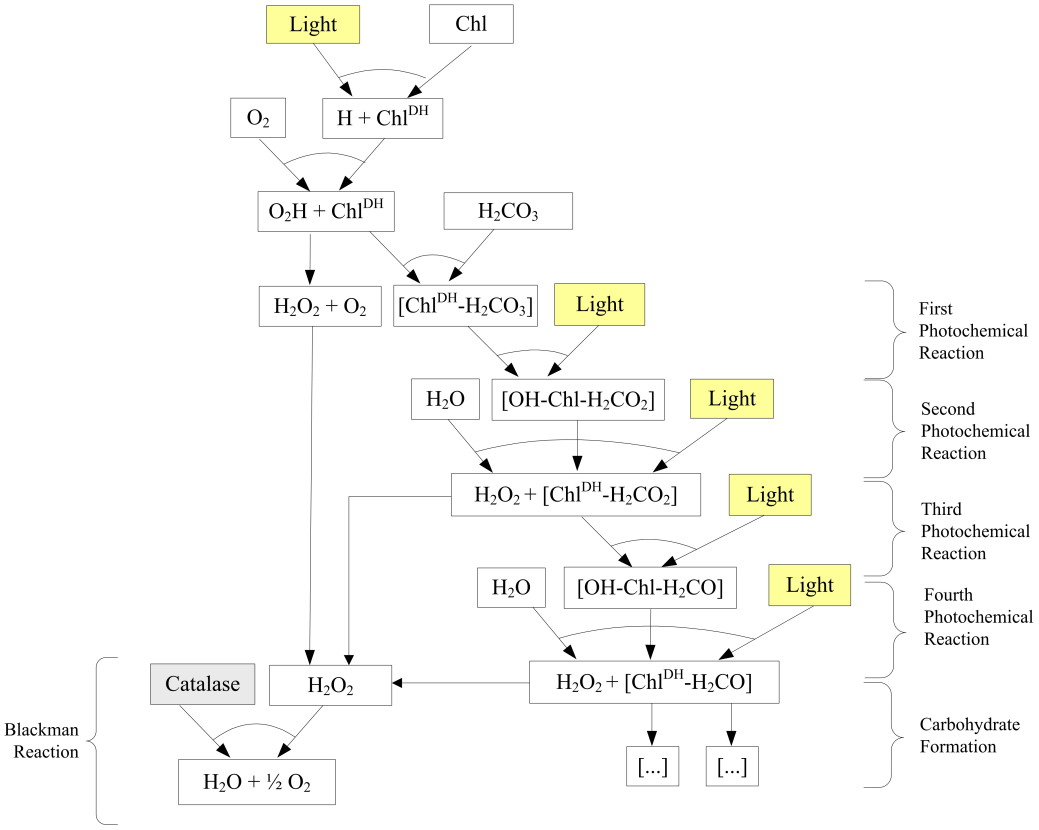

Figure III.4: Franck's model of photosynthesis (1935, 1935b).

and formaldehyde. The formaldehyde then was the usual starting point for the formation of carbohydrates in condensation reactions. If illumination were 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 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.[26]

Franck's suggestion, which is reconstructed in graph form in figure III.4 (121), became widely accepted as the new "standard model" of photosynthesis: an 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 (four 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

[26] Franck (1935*b*), p. 437.

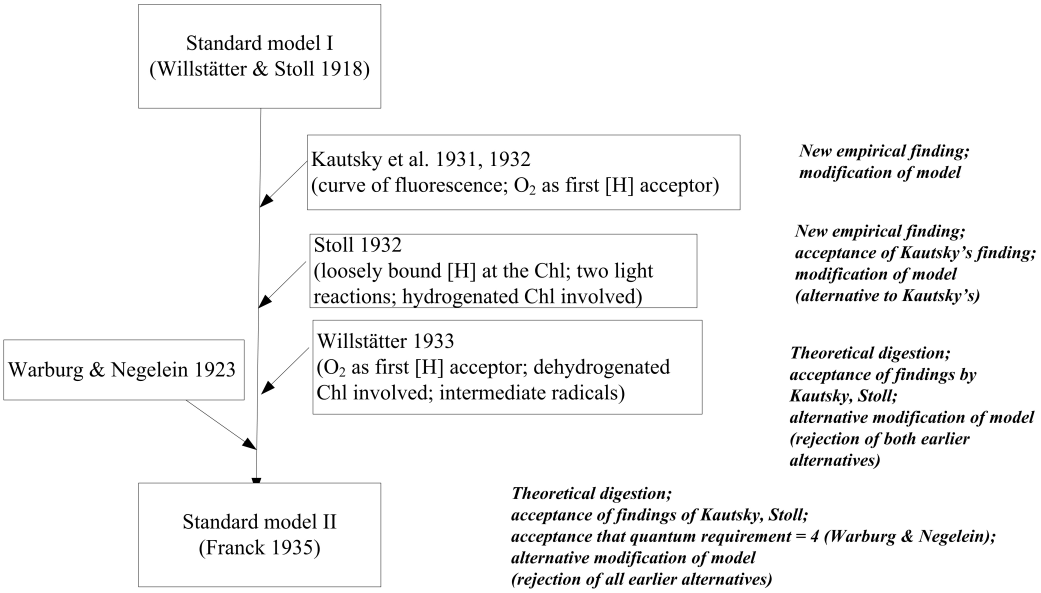

Figure III.5: From the first standard model (Willstätter & Stoll 1918) to the second standard model (Franck 1935).

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 and carbonic acid (or derivatives). Everything seemed to be in place and settled – were it not for the confusing 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 stop to consider the different models described up to this point.

Reflective Interlude

A rough sketch of the relationship between the different proposals is given in figure III.5 (p. 122). The first input considered in this chapter was of an *empirical* nature: based on a recently developed 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 received standard model. Kautsky's group thus suggested that the model be modified: namely 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 received 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 (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) took place. 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 his earlier findings from a theoretical point of view. Willstätter accepted that the new empirical findings (of Kautsky, Stoll and also Warburg and Negelein, on the quantum requirements of photosynthesis) 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, was 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, of what exactly the photochemical reaction of photosynthesis consisted.

Franck's contribution of 1935 marked the final digested state of the model. Franck also accepted the new empirical findings but he took the finding of Warburg & Negelein (1923) – that photosynthesis required only four 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 salvage the phenomena that had been established thus far and, at the same time, the received photosynthesis model.

3 The Photosynthetic Unit: Experiments of 1932

With hindsight, the models of Kautsky, Stoll, Willstätter and Franck were only passing phenomena; 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 in the US. Gaffron and Kurt Wohl, also working in the US, would later provide a theoretical interpretation of the unit (in 1936) as well as coin the actual term.

It all started in 1932 when, in their flashing light experiments, Emerson and Arnold found that, even under optimal conditions, only one molecule of oxygen was evolved in the alga *Chlorella* per about 2400 molecules of chlorophyll.[27] This result was quite unsettling, because up to then it had usually be taken for granted that every chlorophyll molecule would be as active as the other in binding carbon dioxide and, subsequently, reducing it. This had been a fundamental assumption shared by all the photosynthesis models outlined so far in this book. Even Emerson himself had been convinced that the standard model of photosynthesis, in the version that Warburg promoted, was more or less accurate or, at the very least, on the right track – this is evident from Emerson's pre-1932 publications. He had certainly not set out to undermine previous knowledge; rather, his studies with Arnold were motivated by the phenomenon hit upon by Warburg, and earlier by Brown and Escombe, that the attenuation of light by means of rotating sectors did not reduce photosynthesis to the same extent as the attenuation of light by means of filters. This is what they wanted to explore, with the goal of explaining it within the standard framework.[28]

In order to investigate this phenomenon further, Emerson and Arnold developed an elaborated flashing light technique, and ended up with the confusing notion of a large "unit" of photosynthesis, as they already tentatively called it, which consisted of more than 2000 chlorophyll molecules that acted together in order to produce one molecule of oxygen gas. They did not know how to make sense of this finding, which remained inexplicable for the next four years (and even then the interpretation provided by Gaffron and Wohl in 1936 was not immediately well received). In the following sub-section 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.

3.1 Robert Emerson: Harvard, Berlin, Caltech and Stanford

Robert Emerson (see fig. III.6, p. III.6) dedicated his entire professional career to the study of photosynthesis with manometric methods, and his findings profoundly

[27] The experiments and the many difficulties in realising the set-up have been described many times; see, e.g., Myers (1994), Arnold (1991) and Govindjee (2001), second page. See Govindjee, Amesz & Knox (1996*a*) for a special issue of the journal *Photosynthesis Research* dedicated to William Arnold.

[28] See Arnold (1991), p. 74.

Figure III.6: Robert Emerson (1903–59). (Original in the the University of Illinois Archives)

influenced and promoted this field of research.[29] When Emerson first went to Harvard University (Cambridge, Massachusetts) 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,[30] who took on Emerson as his laboratory assistant. 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.[31] 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.[32] With this 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.

[29]The biographical information on Emerson has been taken from the memoir Rabinowitch (1961), complemented by the details given in Govindjee (2004*b*), and by Emerson's own CV of 1936, which is held in his estate: *Curriculum vitae and bibliography of Robert Emerson*, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, University of Illinois Archives.

[30]Cf. Govindjee (2004*b*), p. 184. On Osterhout, see, e.g., Blinks (1974).

[31]Osterhout was, e.g., 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), Osterhout & Haas (1918), Osterhout & Haas (1919) and Osterhout (1919).

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

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.[33] Emerson followed Willstätter's advice, and in 1927 he was awarded his doctorate in Botany from Berlin's Friedrich Wilhelm University for a thesis entitled "On the effect of hydrocyanic acid, hydrogen sulphide and carbon monoxide on the respiration of different algae".[34]" Although the thesis was officially handed in by the university's botanist Hans Kniep, which at first glance implies that Kniep was Emerson's supervisor, 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. Incidentally, Emerson himself felt uneasy about the disciplinary assignment of his thesis. As he wrote in 1949: "I have not been able to live down my embarrassment at obtaining a Ph.D. degree in a subject [Botany] about which I know almost nothing." The main results of this thesis were published in the same year, as Emerson (1927). Its main argument was that, although respiration in *Chlorella* was usually not affected by standard inhibitory agents, this characteristic behaviour changed as soon as the cells were switched from autotrophic to heterotrophic growth by the addition of sugar to the medium. It was a piece of work that could only have originated in Warburg's laboratory, in terms of the technique, the organism and the approach used. It was during the course of his PhD studies and in this laboratory that Emerson became familiar with manometry and *Chlorella* as a model 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 test organism in photosynthesis research.[35]

Emerson then returned to Harvard, where he started working in the laboratory of the physiologist William John Crozier, who had become the head of Harvard's new Laboratory of General Physiology, after Osterhout had left to succeed Loeb in the Department of General Physiology at the Rockefeller Institute in New York City.[36] Emerson stayed in this laboratory at Harvard for another three years, during which he became acquainted with, among many others, Charles Stacy French, then still an undergraduate student. French would later become a postdoctoral student of Emerson's and, finally, Director of the Plant Department of

[33]Govindjee (2004*b*), 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.

[34]Original German title: "Über die Wirkung von Blausäure, Schwefelwasserstoff und Kohlenoxyd auf die Atmung verschiedener Algen. Thesis zur Erlangung der Doktorwürde." Berlin, Oktober 1927, 32 pp.

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

[36]On Crozier and how he became a central figure in general physiology, see Pauly (1987), in particular pp. 201–204.

the Carnegie Institution of Washington.[37] In 1930, Emerson 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),[38] and the plant physiologist Kenneth V. Thimann. This group of young, talented and dynamic scientists were working at the forefront of experimental biology.[39] However, far from being satisfied with the conditions at Caltech, in 1931 Emerson complained in a letter to Crozier about the attitudes of the Caltech biologists, who were all "milk-bottle-molasses and beef-hash-muscle in outlook [...]. The biochemistry section is highly medical in outlook and seems to me very narrow."[40]

However, the intellectual climate at Caltech clearly did not trouble Emerson that much, for he remained there until 1946, only taking a leave of absence in the years 1938 to 1940, which he spent at the Carnegie Institution at Stanford University. From 1946 until his untimely death (in an aircraft crash in 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; and thus one of the most productive and fruitful centres of photosynthesis research came into existence. Emerson's stay in Urbana was only interrupted by a one-year Fulbright Fellowship in 1954, which he spent at the University of Cambridge (UK).

Emerson became one of the leading researchers in photosynthesis. His painstaking accuracy in designing experiments and constructing set-ups, as well as in reading his manometers, 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, that is, he worked mostly with algae. Although it would be interesting to dwell on Emerson's earlier studies and on his personal research pathway, I shall proceed to the work that he undertook with Arnold and that led to the concept of the photosynthetic unit.

3.2 Emerson, Arnold and 2500 Molecules of Chlorophyll

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

[37] On the life and work of French, see, e.g., Govindjee & Fork (2006). French (1979) provides an autobiographical perspective.

[38] Borsook himself remarked that during his studies he had been strongly influenced by Hardolph Wasteneys, who had earlier worked as an assistant to Loeb and Winston Northrop. 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).

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

[40] Quoted in Kohler (1982), p. 322. Emerson to Crozier, 24 March 1931. The original is held by the Harvard University Archives, Pusey Library: Crozier Papers.

in Emerson's class because he could not fit the obligatory course in Elementary Biology into the his timetable. Emerson and Arnold, the professor and the student, 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. 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 seriously considered adding a light source with rotating sectors to his own Warburg apparatus.

Having heard this, Arnold suggested that Emerson might use neon lights instead; 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.[41] In the end, this stay would extend to another fifteen months, and Arnold would never again return to either physics or astronomy. After this project, which led to the first mention of a photosynthetic unit, Emerson encouraged Arnold to do graduate work in biology. Emerson wrote to Crozier about this matter, and Arnold was admitted to Harvard, where he was made a research assistant in the Physiology Department.

Returning to the 1932 experiments, the greatest difficulty the team had in preparing the flashing light set-up 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 millimeters 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 barometer or zero control.

Separating the Photosynthesis Reactions

The first remarkable finding obtained using this set-up, and which was published in 1932, was that if the light period were sufficiently short and the dark period

[41] Arnold (1991), p. 74. See also Govindjee et al. (1996*a*), a special issue of *Photosynthesis Research* dedicated to Arnold.

sufficiently long, photosynthesis rates could be increased by up to 400 per cent.[42] Emerson and Arnold's interpretation of this finding accorded completely with Warburg's earlier suggestion – that the photochemical reaction proceeded rapidly until an equilibrium concentration of its product is reached, which had to be removed by the thermochemical Blackman reaction before the next cycle could start. The important achievement of this first paper of theirs was that it marked the first time that a realistic estimation of the time scale of a full cycle of photosynthesis had been given. 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.[43] These were numerical parameters almost as fundamental as the quantum requirement, which all subsequent photosynthesis models had to accommodate.

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.[44]

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".[45]) 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 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

[42] Emerson & Arnold (1932*b*), p. 417.
[43] Emerson & Arnold (1932*b*), p. 417
[44] Emerson & Arnold (1932*a*), p. 191.
[45] Emerson & Arnold (1932*a*), p. 191.

standard samples of chlorophyll (the material for which, incidentally, had been provided by Hans Gaffron, who at the time was spending a 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 color of the light. The neon light cultures mature faster than the incandescent light cultures, the mercury cultures much more slowly."[46] 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 carbon dioxide reduced per about 2480 molecules of chlorophyll. This implied that *Chlorella* had only one carbon dioxide reducing unit in 2480 molecules of chlorophyll. Emerson and Arnold were completely at a loss as to how to interpret this finding. They were as stunned as their audience, and it would take many years before their result was properly understood.[47] 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.

4 The Generalised Equation for Photosynthesis

4.1 Cornelis B. Van Niel and General Microbiology

Cornelis B. van Niel (see fig. III.7, 131) – 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 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.[48]

Van Niel's first degree, which he was awarded in 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). As microbial fermentation had made up a large share of the curriculum of this subject, his switch to the Microbiology Department for his graduate studies was not that remarkable. When

[46] Emerson & Arnold (1932*a*), pp. 193–194.

[47] The findings were confirmed by Arnold & 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".

[48] On van Niel's life and work, see, e.g., Spath (1999), Barker & Hungate (1990) and Hungate (1986).

Figure III.7: Cornelis B. van Niel (1897–1985).

van Niel first started his studies, the Microbiology Department at Delft was headed by one of the most eminent figures in the field, Martinus W. Beijerinck.[49] In 1921, at the age of seventy, Beijerinck was forced to retire, and Albert J. Kluyver took over as his successor – much to the surprise of Kluyver himself and others in the field, since, up to then, Kluyver had been better known for his chemical expertise than for his knowledge of microbiology. However, his appointment turned out to be fortuitous: Kluyver became the founder of comparative microbiology for which the college in Delft soon became famous. This was the tradition that so enormously influenced the young van Niel.[50]

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; yet only two years later, in 1926, Kluyver published, together with his associate Hendrick J. L. Donker, the classic paper "Unity in Biochemistry".[51] In this paper (which 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.[52] This very much resembled the programme pursued by general physiologists in the US; 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.

In their 1926 paper Kluyver and Donker endorsed Heinrich Wieland's theory of redox reactions as being hydrogen transfers. (As is well known, this notion

[49] See, on Beijerinck's life and work, e.g., Chung & Ferris (1996), van Iterson, den Dooren de Jong & Kluyver (1940) and van Niel (1949).

[50] See, on Kluyver's life and work, e.g., Woods (1957) and Kamp, Rivière & Verhoeven (1959).

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

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

was fiercely opposed by Warburg, who defended the view that oxygen had to be involved in oxidation reactions.[53]) 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 considerable 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 had been.[54]

Such was the intellectual climate in which van Niel received his higher education. Having heard Kluyver's inaugural lecture in 1921, van Niel decided to specialise in microbiology for his last year of study; afterwards he undertook his graduate studies with Kluyver. 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 having been a privilege. While carrying out this work, van Niel used, for the first time, a group of purple bacteria called *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.[55] 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".[56]

Despite the enthusiasm of his student, Kluyver could not be persuaded to accept this as the basis of a doctoral thesis (he believed that the theme was not user-oriented enough, and that it was unlikely to be successful). 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 cheeses. Kluyver insisted that this would be a much better preparation for the work in industry that Kluyver anticipated for his students. Furthermore, Kluyver believed that, as purple bacteria grow 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

[53]See, e.g., Werner (1997) for a discussion of the ensuing controversy between Warburg and Wieland.

[54]See Spath (1999), Ch. 1, pp. 36–37.

[55]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.

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

of the holes in Swiss Emmental cheese. Albeit amusing, this was definitely not the kind of fundamentally important microbiology in which van Niel hoped to engage.

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 accordance with Kluyver) that it was inappropriate to associate it, 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.[57] Van Niel 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; van Niel would work for the next thirty-five years of his life in the Jacques Loeb Laboratory of this station.

At the time, experimental biology was being strongly promoted at Stanford University (as at many other institutions in the US). It was a field that had started to thrive not least because it was being fostered by the Rockefeller Foundation.[58] The establishment of the new laboratory at the marine station was part of a general shift to experimental biology. Emulating, perhaps, the successful profile of the Zoological Station in Naples (Italy), the laboratory was not organised along disciplinary boundaries[59] and thus brought together proponents of very different branches of biology, pursuing a number of different research themes. The young biophysicist L. G. M. Baas Becking was head of the laboratory; 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 an entirely 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

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

[58] 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.

[59] See, on the *Stazione Zoologica di Napoli*, e.g., Groeben (1975), Fantini (2000) and Groeben (2005).

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

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 in the next section.

4.2 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; he gave a talk at a gathering of the Western Society of Naturalists, which that year was holding its traditional winter meeting in Pacific Grove. His 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. One has to recall that in 1930 photosynthesis was still defined as a process in green plants that produced oxygen – which *by definition* excluded the possibility that photosynthesis might occur elsewhere. Van Niel now set out to persuade people that the process familiar to plant scientists was only one out of a whole range of possible instantiations of a general formula. This was going even further than the earlier suggestion that had been proposed by the French plant physiologist René Wurmser – that the oxygen produced during photosynthesis might come from water.[62] In 1935 van Niel gave a succinct summary of his findings (given in the form of ten points), which is worth quoting *in extenso*:

> (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 oxidized and the amount of CO_2 which has disappeared, to wit: for one molecule of H_2S oxidized to S, 0.5 molecule

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

[62] See, e.g., Wurmser (1921), Wurmser (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.

of CO_2 disappears (green bacteria); for 1 mol. of H_2S oxidized 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.[63]

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 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:

$$2CO_2 + H_2S + 2H_2O \xrightarrow[\text{light}]{} 2[CH_2O] + H_2SO_4 \tag{III.6}$$

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

$$CO_2 + H_2O \xrightarrow[\text{light}]{} [CH_2O] + O_2 \tag{III.7}$$

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

$$CO_2 + 2H_2A \xrightarrow[\text{light}]{} [CH_2O] + 2A + H_2O \tag{III.8}$$

In this form, photosynthesis was understood to be the photochemically driven reduction of carbon dioxide with a variety of hydrogen donors. Of course, this broad conception also bore enormous consequences for the understanding of photosynthesis in plants. Van Niel himself addressed this question at length 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,

[63] Van Niel (1935), pp. 138–139.

> 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.[64]

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 four 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.[65]

As one might expect, researchers in photosynthesis were not yet ready to accept van Niel's revolutionary 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. Indeed, it 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, together with most of 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 and 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 contemporaries. Even in Marjorie Stephenson's 1930 seminal monograph *Bacterial Metabolism*, the author did not include the mechanism of photosynthesis in the many different microbiological pathways that she examined. 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 believed 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

[64] Van Niel (1935), pp. 142–143.
[65] Van Niel (1935), p. 143.

> 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".[66]

Although this does surely not give justice to the enormous 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"), 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 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.[67]

Van Niel's own achievements were the most compelling evidence for this bold and sweeping statement. He provided a new and utterly unexpected link between general microbiology, general physiology and general biochemistry; and it was his personality that made this link appear even more compelling and that 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 become a thriving research centre for this discipline. In the next section, however, I shall take a look at the work of a contemporary scientist, who at first strongly opposed van Niel but then eventually became one of the latter's staunchest supporters and, for a time, close collaborator: 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 chlorophyllous plants.

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

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

5 The Conceptualisation of the Photosynthetic Unit (1936)

Hans Gaffron spent the first ten years of his life in Lima, Peru, where his father Eduard had settled as an affluent physician.[68] 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 entitled "Beiträge zur Kenntnis der Ester der Sulfamidsäuren" ("Contributions to the knowledge of the ester compounds of sulfamide acids"), which he completed at the Chemical Institute of Berlin's Friedrich Wilhelm University under the supervision of Wilhelm Traube.[69] 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; 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, which testifies to the good working relationship they must have had. In 1931, Gaffron went to Caltech in the US 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,[70] 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. When, in 1940, Gaffron was asked by the Federal Bureau of Investigation (FBI) why he had left Germany, Gaffron replied as follows:

> In the course of less than two years [after the Nazis had come to power] the Kaiser Wilhelm Institute became the only place where I could hope to proceed with scientific work without being molested by Nazi party regulations. Submission to such regulations became a *conditio sine qua non* for a university career. Though not interested in active politics I was against Nazism in any form from its earliest beginning because its doctrine opposes liberalism, democracy, and free scientific research. [...] When it became clear that the Hitler government would remain and grow in ferocity – with war

[68] 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).

[69] I am grateful to the historian of science Phillip Sloan, of the University of Notre Dame, Indiana (US), for sharing this information with me.

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

> as the eventual unavoidable outcome – I arranged for my emigration from Germany.[71]

Thus, at the end of 1937, Gaffron and his wife left for the US and took refuge in the laboratory of his former adversary van Niel 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 six months of his stay at the marine station.[72] 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 twenty years of his life.

Bacterial photosynthesis was to become Gaffron's main research theme; and 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 this field in 1933 with a study of 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*).[73] The interpretation of some of his data on the metabolism of purple bacteria sparked off much contention – on the question as to whether or not organic substances could serve as hydrogen donors in purple sulphur bacteria – between Gaffron and van Niel. While van Niel (1931) had purported that this was the case, Gaffron (1934) claimed to have found evidence to the contrary. This was countered in Van Niel (1935), with the conjecture that Gaffron had used cultures that were contaminated, which understandably infuriated Gaffron. 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. Gaffron checked this out with his own strains of bacteria and

[71] Quoted in Rürup (2008), p. 200.

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

[73] Gaffron (1933*b*), p. 2. Note the close parallel between Gaffron's work and French's studies during these years. In French (1937), p. 71, the latter wrote, e.g.: "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."

[74] See Homann (2002), p. 94.

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.

5.1 Context and Scope of the 1936 Paper

The contribution to 1930s 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.[75] In it, the two young men set out to argue why the standard model of photosynthesis was no longer tenable. (Note, however, that at the time Gaffron had not yet accepted van Niel's generalised equation for photosynthesis!) In view of the results of the Emerson–Arnold experiments, 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 propositions had already been made three years earlier in Gaffron (1933*a*); 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 physicist Max Delbrück in Berlin that these ideas had been elaborated and used as the basis for a new concept of photosynthesis.[76] This seminar was the very discussion circle that had produced, in 1935, the celebrated paper "On the Nature of Gene Mutation and Gene Structure", co-authored by Delbrück, Nikolai W. Timofeeff-Ressovsky and Karl G. Zimmer, which is also known as the "Three Man Paper" (or 3MP) and is often regarded as the stimulus behind what would later become the discipline of molecular biology. Thus, the whole context is worth a little further consideration.[77]

Delbrück, who originally trained as a physicist, had gone to Berlin in 1932 to work at the KWI for Physical Chemistry as an assistant to the Austrian-born physicist Lise Meitner. Before that, Delbrück had spent some months in Copenhagen with Niels Bohr, who was elaborating on the deeper meaning of quantum mechanics – in particular, the complementarity principle. Later Delbrück recalled that, even then, 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 re-interpretation from the viewpoint of physics.[78] In Berlin, Delbrück decided to explore, together with like-minded scientists, the

[75] Gaffron & Wohl (1936).

[76] See Gaffron & Wohl (1936), p. 81.

[77] Timofeeff-Ressovsky, Zimmer & Delbrück (1935). For information on the Delbrück seminar and the development of biophysics in Berlin at the time, see Sloan (2009), which Phillip Sloan generously made available to the author prior to publication. Phillip Sloan personally communicated to the author that he is working on a more detailed study of the impact of the Delbrück seminar on the general development of biophysics, which also influenced the development of photosynthesis.

[78] See the interview with Delbrück, carried out as part of the Caltech Archives Oral History Project, for the latter's recollections of these years; *Max Delbrück: Oral*

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 left for the US). 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 [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.[79]

Thus, even so many years later, Delbrück still vividly remembered that the discussions had centred on photosynthesis. Delbrück was mostly 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 interests 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 autobiographical account 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 outside the laboratory, "except for a few seminars on photosynthesis at Max Delbrück's house with Hans Gaffron and Eugene Rabinowitch".[80] Although these may have been private discussions that were not part of the "official" Delbrück seminars, it would be more constructive to consider them within the same context. Thus, French and Rabinowitch should also 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, published in two halves as Gaffron & Wohl (1936). This was followed, four years later, by a comprehensive review of the same problem in English by Wohl.[81] 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: the data obtained in biological systems were systematically and carefully subjected to an interpretation from a quantum physics point of view. I shall now examine their arguments.

History Interview with Carolyn Harding, July 14-September 11, 1978, CIT, p. 55, <http://resolver.caltech.edu/CaltechOH:OH_ Delbruck_ M> (n.d.), pp. 41ff.

[79] Oral History Interview with Carolyn Harding, p. 55.

[80] French (1979), p. 7.

[81] Wohl (1940).

5.2 Critique of the Standard Model

Gaffron and Wohl began their paper with an outright rejection of the 1935 standard model, 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, and which eventually led to the formation of formaldehyde and hydrogen peroxide – according to the following equation:

$$CO_2 + 3H_2O \xrightarrow[\text{light}]{} CH_2O(\text{Formaldehyde}) + 2H_2O_2 \tag{III.9}$$

Gaffron and Wohl pointed out that this pathway was impossible, since the reaction of carbon dioxide to formaldehyde required more energy than the four light quanta, which were needed for photosynthesis to take place, could provide. 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 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. Gaffron and Wohl could not see how one could possibly reconcile these requirements: "Unfortunately, one usually has to choose between these three desired properties: high yield, low activation energy, long lifespan."[82] Furthermore, Gaffron and Wohl pointed out that the H/OH exchange mechanisms suggested by Franck led to even more problems, including back reactions and unstable intermediates.

Gaffron and Wohl then turned to the next problem of the standard hypothesis – that chlorophyll was thought to be an actual raw material of the reaction. 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. The fact that chlorophyll had two "loose" hydrogen atoms in its structure, which seemed to make it very likely that chlorophyll itself reduced the carbon dioxide (by donating these hydrogen atoms) also failed to convince the authors; they cited evidence that, although these loose hydrogens were responsible for the high autoxidation rate of chlorophyll, they did not seem to be at all connected to the photosynthetic reactions.

And what about the specific role of magnesium? Gaffron and Wohl stated that magnesium almost certainly contributed to the chlorophyll's green colour and strongly influenced the absorption spectrum; they also believed that it had a role in stabilising the system of double bonds and other parts of the molecule. However,

[82] See Gaffron & Wohl (1936), p. 82.

Gaffron and Wohl 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. In general, if it were accepted that carbon dioxide was first bound by some cell constituents before it moved to the site of reduction (namely the chlorophyll molecule), this would imply a very strong affinity for chlorophyll to carbon dioxide – even though it had been experimentally shown some time ago that the affinity for chlorophyll to carbon dioxide was rather low. Although Gaffron and Wohl admitted that there might be an unknown derivative that was bound to chlorophyll instead (as Willstätter and Stoll had suggested), they drew attention to the fact that no evidence had so far been produced in favour of this option. It had been proven that carbon dioxide was actually present in photosynthesising cells in a chemically bound form, yet most probably not in a complex with chlorophyll.

5.3 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 four 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 four light quanta, as set out in the standard model. Under the conditions chosen by Warburg and Negelein, their result was negative:

> According to the Franck–Stoll theory photosynthetic assimilation should, at the given light intensity, become noticeable only after about ten minutes – since one can calculate that 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. By contrast, Warburg and Negelein found a yield that approximated the theoretical maximum![83]

The solution, the authors maintained, could be found in the experiments carried out in 1932 by Emerson and Arnold, who had found that the light saturation point of photosynthesis was reached at an intensity that reduced one molecule of carbon dioxide per (roughly) every 2500 molecules of chlorophyll. Emerson and Arnold had called the set of 2500 pigment molecules "one unit", Gaffron and Wohl reported, although they had not gone into any detail as to how this "unit" should be understood or whether all those 2500 molecules were part of the active "unit". Gaffron and Wohl were more courageous and jumped right in to fill this gap. Their suggestion was as follows:

> We want to assume that almost all of these 2500 chlorophyll molecules are part of one unit, and that they are, when compared with each other, totally equivalent. 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". According to this concept, the "unit" cannot possibly be

[83] See Gaffron & Wohl (1936), p. 86.

> reconciled with the Stoll–Franck theory; yet it nicely explains the excellent quantum yield in Warburg and Negelein's experiments. If, in the course of the experiment, one chlorophyll molecule receives on average 0.8 [light] quanta, this means that 2500 chlorophyll molecules receive, in the same time, about 2000 [light quanta]. One molecule of carbonic acid thus receives the four quanta necessary for its reduction in, on average, about one second!"[84]

In the next section of the Gaffron–Wohl paper this theory was tested and the concept of a "unit", as suggested by Emerson and Arnold, was confirmed, albeit in a totally different way.

5.4 Test and Confirmation

As their starting point, Gaffron and Wohl took Emerson and Arnold's experimentally based estimation of the Blackman reaction's duration. This was interpreted by the authors as follows:

> We assume that it has been safely established that, on average, the actual reaction rate τ of the Blackman reaction is 0.02 seconds at 25°C. 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 (i.e. in the form of an assimilatory apparatus), must receive in these 0.02 seconds, at the given stationary [light] intensity, four light quanta. However, under the experimental conditions, delineated in Table I [the conditions used in the pertinent experiments], one molecule of chlorophyll in green leaves (or *Chlorella*) receives its four quanta only in twenty seconds. It follows from this that approximately 1000 molecules of chlorophyll must act together to supply one molecule of carbonic acid in 0.02 seconds. Thus, we arrive at the postulation of an assimilatory unit of about 1000 *active* chlorophyll molecules.[85]

The authors emphasised that they had reached this conclusion by following a completely different path to the one that Emerson and Arnold had chosen. Their respective results were also not quite identical: Emerson and Arnold had found that, for each carbon dioxide molecule to be assimilated, 2500 chlorophyll molecules were, in general, at its disposal; Gaffron and Wohl had been able to ascertain the exact number of chlorophyll molecules that were actively involved in the reduction process of this one carbon dioxide molecule – 1000. In view of these roughly coinciding results, arrived at by totally different means, Gaffron and Wohl considered it as proven that the photosynthetic units were real entities.

Gaffron and Wohl suggested that the differences between "active" and "inactive" chlorophyll molecules might explain the apparently confusing results arrived at by Willstätter and Stoll – that photosynthesis was not proportional to the chlorophyll content of the cell. Gaffron and Wohl raised the possibility that this variation might be connected to the way that chlorophyll molecules acted in concerted units. Thus, the cell's chlorophyll could be less efficient if, for example, some excess chlorophyll were present and not coordinated to a unit or if the number of chlorophyll molecules required for a unit were simply variable. The authors

[84] See Gaffron & Wohl (1936), p. 87.
[85] See Gaffron & Wohl (1936), p. 88.

considered the latter hypothesis the most interesting. Photosynthesis should work more efficiently in cells with very small units than in cells with large units.

The mechanism underlying this cooperative action 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 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 clearly 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 four light quanta were required, one had to be extremely parsimonious with the energy expenditure in modelling the mechanism. The involvement of high-energy intermediates, such as peroxides, was out of the question. Gaffron and Wohl could offer no convincing alternative to the underlying chemical pathway, yet their proposal – of highly efficient, concerted units of chlorophyll molecules – was at least able to explain the short induction period of the process.

6 Franck and Herzfeld's Conservative Alternative

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 very long-lived – too long-lived to provide a realistic option. In his subsequent papers Franck himself added a number of observations that criticised his earlier model; while in 1937, Franck published a paper co-authored with the physicist Karl F. Herzfeld, in which he tackled the problem once more and set out to contest the view that recent experiments "make the assumption of a photosynthetic unit necessary, in which a large number of molecules cooperate in a way not encountered *in vitro*".[86] 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 a colloidal but in a unimolecular state (since colloidally resolved chlorophyll *in vitro* did not emit any fluorescence). 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 four 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 peracid, formic acid and a peraldehyde, which, as Franck and Herzfeld did not fail to mention, "are the same intermediate

[86] Franck & Herzfeld (1937), p. 238.

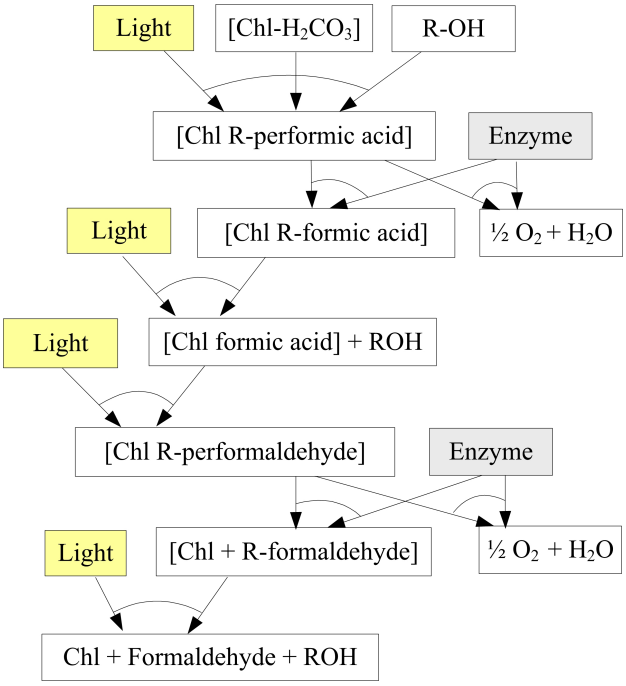

Figure III.8: The model of Franck & Herzfeld (1937).

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".[87]

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."[88] This idea was introduced in order to avoid the notion of the existence of free radicals, which, according to Willstätter and others, were also present in abundance, but 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".[89]

The mechanism is reconstructed in graph form in figure III.8 (p. 146). Four light reactions corresponded to the four 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

[87] Franck & Herzfeld (1937), p. 237.
[88] Franck & Herzfeld (1937), p. 240.
[89] Franck & Herzfeld (1937), p. 239.

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.[90]

Franck and Herzfeld admitted that even they did not believe that this model constituted the final solution. However, they did maintain that, as it explained so many phenomena, it was a good working hypothesis.[91] 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 proposition 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, among them Emerson's own interpretation: in Emerson (1936) he 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 about 1/2500 of the chlorophyll.[92] Optical models, which tried to fill in the gaps of the "unit" concept, were also proposed, beginning with two papers by Wohl.[93] In these models it was assumed that there was a rapid transfer of absorbed energy from excited chlorophyll molecules to so-called reaction centres (of unknown material nature and one per several thousand molecules of chlorophyll) to which the carbon dioxide was attached. In short, the general state of the discussion 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.[94]

[90] Franck & Herzfeld (1937), p. 240.

[91] See Franck & Herzfeld (1937), p. 241.

[92] Emerson (1936). In his obituary of Emerson in 1961, Rabinowitch still considered this to be 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".

[93] Wohl (1940) and Wohl (1941).

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

7 Franck and Herzfeld Again

In the years that followed Franck used a number of 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". At first glance, the paper seems to have nothing to do with photosynthesis, although on closer inspection one realises that Gaffron and Wohl's explanation of the energy transfer in a photosynthetic unit directly stimulated the work.[95] 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.[96] Energy absorbed at any point of this crystal would then migrate through the crystal and be channeled towards the carbon dioxide molecules. Franck and Teller maintained that this was highly improbable, because the migration of excitation energy would be bound to trigger a much higher level of fluorescence in photosynthesising leaves than was actually the case.[97]

By 1941, Franck had discarded the model he had suggested with Herzfeld in 1937 and replaced it with a new version, not because of any inherent weaknesses of the first attempt, Franck and Herzfeld explained, but because of decisive new developments in the field which had made most of their earlier work obsolete.[98] Most importantly, by the end of the 1930s, a number of photosynthesis researchers working in the US had started to doubt the validity of Warburg and Negelein's proposal that four light quanta were the minimum requirement for photosynthesis. A value of ten to twelve light quanta seemed to be more realistic, and this boost of the energy budget (by the factor two to three!) radically changed and, in fact, greatly alleviated, the task of modelling the process (the ensuing controversy is discussed in Chapter IV). Furthermore, Franck and Herzfeld referred to the work undertaken by Samuel Ruben, Martin Kamen and their co-workers, who, with the help of radioactive carbon isotopes, had found that carbon dioxide reacts with an acceptor molecule, RH, in a carboxylation process, the result of which was the formation of RCOOH, whose concentration was of the same order as the chlorophyll in the solution (see Chapter V for a discussion of Ruben and Kamen's work). And, finally, Franck and Herzfeld cited work on the fluorescence of photosynthesis, at least partly carried out by Franck himself and his co-workers; they had found, for example, that the addition or removal of carbon dioxide produced changes in the rate of fluorescence that corresponded exactly to changes in the rate of photosynthesis. Franck and Herzfeld saw this as a strong indication of the fact

[95] Franck & Teller (1938), p. 861.

[96] Note, again, 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).

[97] On the paper's argument, see also the review Franck & Gaffron (1941), p. 210. Note, however, that Franck and Teller assumed that there existed a one-dimensional structure (a linear chain of chlorophylls); the application of two- or three-dimensional models, in, e.g., Bay & Pearlstein (1963), led to very different results. Robinson (1967) shows clearly where Franck and Teller went wrong, while an alternative model was first elaborated in Pearlstein (1966) and Pearlstein (1967). See Pearlstein (2002) for a short review of this theme.

[98] See Franck & Herzfeld (1941).

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."[99]

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 by Ruben et al. and referred to above 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, RCOOH, 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 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.[100]

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.[101]

The catalyst responsible for stabilising the reaction was called "B". Franck and Herzfeld believed that each catalyst B molecule stabilised only one molecule of

[99] Franck & Herzfeld (1941), p. 979.
[100] Franck & Herzfeld (1941), p. 982.
[101] Franck & Herzfeld (1941), p. 985.

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:

$$\begin{aligned} \text{Chl-RCOOH} &\xrightarrow[\text{light}]{} \quad \text{Chl'}_1 \\ \text{Chl'}_1 + \text{Catalyst B} &\longrightarrow \quad \text{Chl*}_1 + \text{Catalyst B} \end{aligned} \tag{III.10}$$

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.

8 Isolated Chloroplasts and Water Splitting

8.1 Robert (Robin) Hill and the Chloroplast Reaction

The biochemical work done by Robert (or "Robin" as he was usually called by friends and colleagues) Hill[102] (see fig. III.9, p. 151) 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.[103] 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 Rudolph Peters at the University of Oxford and by David Keilin at the Molteno Institute, Cambridge) was a notable exception. 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. As early as his celebrated 1913 lecture to the British Association for the Advancement of Science, 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.[104] Hopkins even used this as an argument for introducing the study of biochemistry as an independent discipline, which frequently met with the objection that it was too narrow a field of study:

[102] 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.

[103] On Hopkins and his institute, see, e.g., Needham, Dunn & Baldwin (1949) and, more recently, Kohler (1982), Chapter IV, pp. 73ff.

[104] See Hopkins (1949).

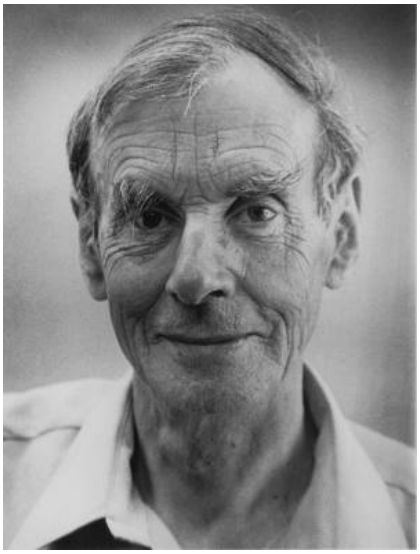

Figure III.9: Robert (Robin) Hill (1899-1991).

> The recognition of Biochemistry as a specialised departmental subject is mainly justified [...] by the fact that in the study of metabolism it provides a common ground and a common technique for biologists whose special interests and ultimate activities may greatly vary.[105]

When Hill arrived (in 1919) as an undergraduate student at the University of Cambridge to specialise in chemistry (he was also deeply interested in plants), Hopkins's department was on the point of entering a period of enormous expansion.[106] While in 1920 the Hopkins group counted ten workers, by 1925 this number had soared to fifty-nine.[107] Hill was encouraged by his fellow student and close friend Malcolm Dixon to join Hopkins's Biochemical Laboratory. 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 type".[108] Hill had originally intended to embark on a serious, biochemical study of natural dyes and plant pigments, a subject that had engrossed him for some time already.[109] 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.

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[105] Quoted in Kohler (1982), p. 75.

[106] 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.

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

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

[109] 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.

In 1924, Hopkins's Biochemical Laboratory moved into its new (and now famous) building in Tennis Court Road, thereby coming into the immediate vicinity of the Molteno Institute, where, in the years 1920 to 1925, Keilin carried out his seminal studies of cytochromes.[110] 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*.[111] Hill collaborated 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 VI).

Keilin can justifiably be called the master of cytochromes. He came to this research theme somehow accidentally through his investigations of peculiar haemoglobin phenomena in the larva of a certain 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; he rediscovered a compound that had already been described in 1886 by the Englishman Charles A. MacMunn. Yet it was Keilin who 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.[112] 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 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.[113] 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

[110] Keilin was made director of the institute in 1931. On Keilin's life and work, see e.g., Mann (1964). Keilin started his career with definitive organismal interests (in animals; more specifically, beetles), and became a proficient entomologist; yet, as his biographer puts it: "He realised rather early that morphology alone would be insufficient in studies on the evolutionary and adaptation phenomena [...]. His attention was gradually turning towards cellular physiology and biochemistry as a means of advancing his research." However, even during the years of his research into cytochromes, Keilin never gave up his pursuit of questions on the morphology and physiology of insects. Mann (1964), p. 186.

[111] The results of this collaboration are the papers Keilin, Dixon & Hill (1931) and Keilin & Hill (1933).

[112] Keilin (1925*b*), p. 315.

[113] Keilin (1925*a*). See, e.g., Keilin (1966) on the history of research into cell respiration and cytochromes.

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. He embarked on a study of this compound's reversible oxygenation, during which 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.[114] 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 he could move. In 1936, Hill finally "started a series of experiments designed to obtain light-induced oxygen evolution with chloroplast preparations freed from the leaf".[115] With hindsight, Hill 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.[116]

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? However, 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 at least from the time of 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.

His persistence paid off. By ingenious, albeit at first glance somewhat primitive, 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.

[114] Hill (1965), p. 124.
[115] Bendall (1994), p. 153.
[116] Bendall (1994), p. 153.

(Note that 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. Hill mixed his chloroplast suspension with a solution of myoglobin, under air-tight conditions, and observed spectroscopically the conversion of haemoglobin into oxymyoglobin at high light intensities.[117]

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 Fe^{III} ions (ferric 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 solutions. This was, at first glance, 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.

In his 1937 publication, Hill carefully avoided jumping to any rash conclusions – he was keenly aware of the possibility of experimental artefacts. On a practical level, carrying out the actual experiments was immensely complex and required enormous skill and circumspection, for example, in order to ensure that the production of oxygen was not due to some property of the haemoglobin, which he extensively discussed in Hill (1939). Another point of concern was the fact 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 means of demonstrating a general property of the chloroplast.[118] It seemed that ferric oxalate or other

[117] See Hill (1937) and Hill (1939) for the first seminal 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 abbreviated to "haemoglobin".

[118] See Hill (1939), p. 207.

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:[119]

> 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:
>
> $$\begin{aligned} 2\mathrm{A} + 2\mathrm{H_2O} &\longrightarrow \quad 2\mathrm{AH_2} + \mathrm{O_2} \\ 2\mathrm{AH_2} + \mathrm{B} &\longrightarrow \quad \mathrm{BH_2} + \mathrm{A} \end{aligned} \tag{III.11}$$

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".[120] 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.[121] 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."[122] 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.[123]

This reaction – the production of oxygen by chloroplasts in solution supplied with artificial hydrogen acceptors – later became known as the "Hill reaction",

[119] Hill (1939), p. 207.
[120] Hill (1939), p. 209.
[121] See Hill & Scarisbrick (1940*b*) and Hill & Scarisbrick (1940*a*).
[122] Hill & Scarisbrick (1940*a*), p. 61.
[123] Hill & Scarisbrick (1940*b*), p. 254.

a term that was coined in 1941 by Charles Stacy French and 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."[124] 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.[125] 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.[126] 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".[127] The paper was not exactly a sweeping success. As Myers later recalled: "It was greeted by a rather stony silence."[128] Some years were to pass before the importance and accuracy of Hill's findings would be realised.

8.2 Implications of the Findings

Hill's two main contributions to photosynthesis research were: first, that he succeeded in separating the photosynthetic production of molecular oxygen from the reduction of carbon dioxide to carbohydrates, and, by doing so, provided convincing evidence that these two parts of photosynthesis occurred separately; and, second, his findings suggested that the photochemical part of photosynthesis comprises 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 from a totally different starting point) that the photosynthetic oxygen originates from the light-induced hydrogen transfer from water to an appropriate acceptor. Hence, water, and not carbon dioxide, was the source of photosynthetic oxygen, although carbon dioxide was, presumably, reduced in a thermochemical process. And although these consequences were not immediately understood, nor indeed accepted by Hill's contemporaries, they nevertheless had a marked effect on the field. As Gaffron wrote in an 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

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

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

[126] French (1979), p. 10; see French & Anson (1941) for the abstract of the paper. Note that in the autobiographical accounts of this episode in French (1979) as well as in Myers (1974), the name of the society was inaccurately reported.

[127] French & 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.

[128] Myers (1974) p. 422.

> Hill's chloroplast reaction and later my photoreduction experiments made any other than van Niel's view untenable was I ready to give in.[129]

Besides these conceptual consequences concerning the mechanism of photosynthesis, Hill's achievements opened up completely new avenues in methodology. Hill was the first biochemist 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 Fe^{3+} to NADP.[130] 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.

9 On the Verge of New Perspectives

Biological Studies Generalised

The general goal of most of the actors in this chapter was in no way different to the goals pursued by the experimental biologists of earlier periods: to explain how the metabolism of organisms works. Yet, while it had began to dawn on biologists working around 1900 that this goal would not be reached without the input of physicists, chemists and mathematicians, it was only by the late 1920s and early 1930s that this insight began to be reflected at academic institutions.[131] If goals are to be reached, they require appropriate means; and if the latter – such as a deep general knowledge of all the natural sciences for the pursuit of biological research – are not at hand, then the sub-goal of realising these means takes priority. The period examined in this chapter shows strikingly convergent developments in the different fields of experimental biology, such as physiology, biochemistry and microbiology, which illustrate the firm conviction held by researchers that physical and chemical tools, concepts and methods were indispensable for studying life processes (and, hence, had to be included in the curricula) and for 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 model organisms in the study of the metabolism of higher organisms. Biochemical unity at the metabolic level became part of the body of generally accepted knowledge.

The atmosphere of change is also clear when one looks at the general development of the collective as a whole. Far more people than ever before became interested in photosynthesis; new sub-goals 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 the ramifications of more photosynthesis research being undertaken, one can observe a marked increase in the frequency and popularity of

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

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

[131] As early as 1897, Wilhelm Pfeffer had maintained that the most difficult problem in all of science was to reach an appropriate understanding of the physiological details of organisms and that progress could only be made if, first of all, simplistic model situations were studied, and if chemists and physicists supported the project with their expertise. See Pfeffer (1897), Introduction.

conferences and more informal meetings on the subject. Many people strongly felt that the problem was much more complex than had previously been envisaged and that a multi-dimensional 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 Group at the University of Illinois at Urbana–Champaign, headed by Robert Emerson and Eugene Rabinowitch; the Photosynthesis Laboratory at the University of Chicago, led first by James Franck and then later by his long-standing collaborator Hans Gaffron; and, starting in 1945, 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 sections to this chapter, the 1930s also saw the appearance of the first "professional" researchers in photosynthesis – scientists who developed more than a mere 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 II; Emil Warburg's work most probably 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). Robert Emerson, William Arnold and Charles Stacy French were all trained in 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. Note that during the 1930s a number of totally different disciplines greatly enhanced and fostered the application of physical and chemical methods to biological problems; it was, for example, in this decade that Warren Weaver launched the Rockefeller Foundation's programme to support projects in the field that he, at the time, called "molecular biology".[132]

This general intellectual climate was obviously a good preparation for a successful career in photosynthesis studies, which was frequently strengthened by the close interpersonal links between the players – the second related factor. Gaffron, for example, came to photosynthesis by way of Otto Warburg (he worked as the latter's assistant). Gaffron's interest was strengthened by the discussions he held with Max Delbrück, Kurt Wohl and other physicists, 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,

[132]Of the wealth of literature on this topic, see, in particular, the already classic studies Kohler (1991) and Kay (1993).

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. The close interpersonal links between the actors clearly played a considerable role in attracting scientists to photosynthesis and enhancing interest in the field.

On summarising these observations, one would be entitled to state that research in 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 "new" or molecular biology, which prior to then had perhaps been too frequently regarded as being equivalent to molecular genetics.[133]

The Main Lines of Thought

We have found that, in the 1930s, scientists approached photosynthesis from a number of very different angles and traditions, and that these approaches, at least in their early stages, developed mostly independently of each other, so that the main sections of this chapter could be presented as blocks in their own right. Figure III.10 (p. 160) provides an overview of the models and their relationships to each other. With the exception of van Niel and Hill, who came from rather different scientific backgrounds, the scientists reacted closely to their colleagues' work, which clearly influenced the models discussed in this chapter. Section 2.3 looked at the relationships between the models leading up to Franck's 1935 suggestion. Gaffron and Wohl harshly criticised this proposal, upon which Franck & Herzfeld (1937) attempted to find a better solution. Franck & Herzfeld (1941) then initiated a new era of models, which were constructed under the assumption that far more than four light quanta were available for the completion of the photochemical process. I shall now summarise the general development and the rationale behind each of the main lines of research.

Fluorescence Studies and a New Standard Model

The first line of thought continued the tradition that was outlined in Chapter I. 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 five 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, while 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, together with his collaborator E. Wiedemann, Stoll turned again to chlorophyll – to its structural properties as well as other

[133] See, e.g., the argument brought forward in Zallen (1993*b*).

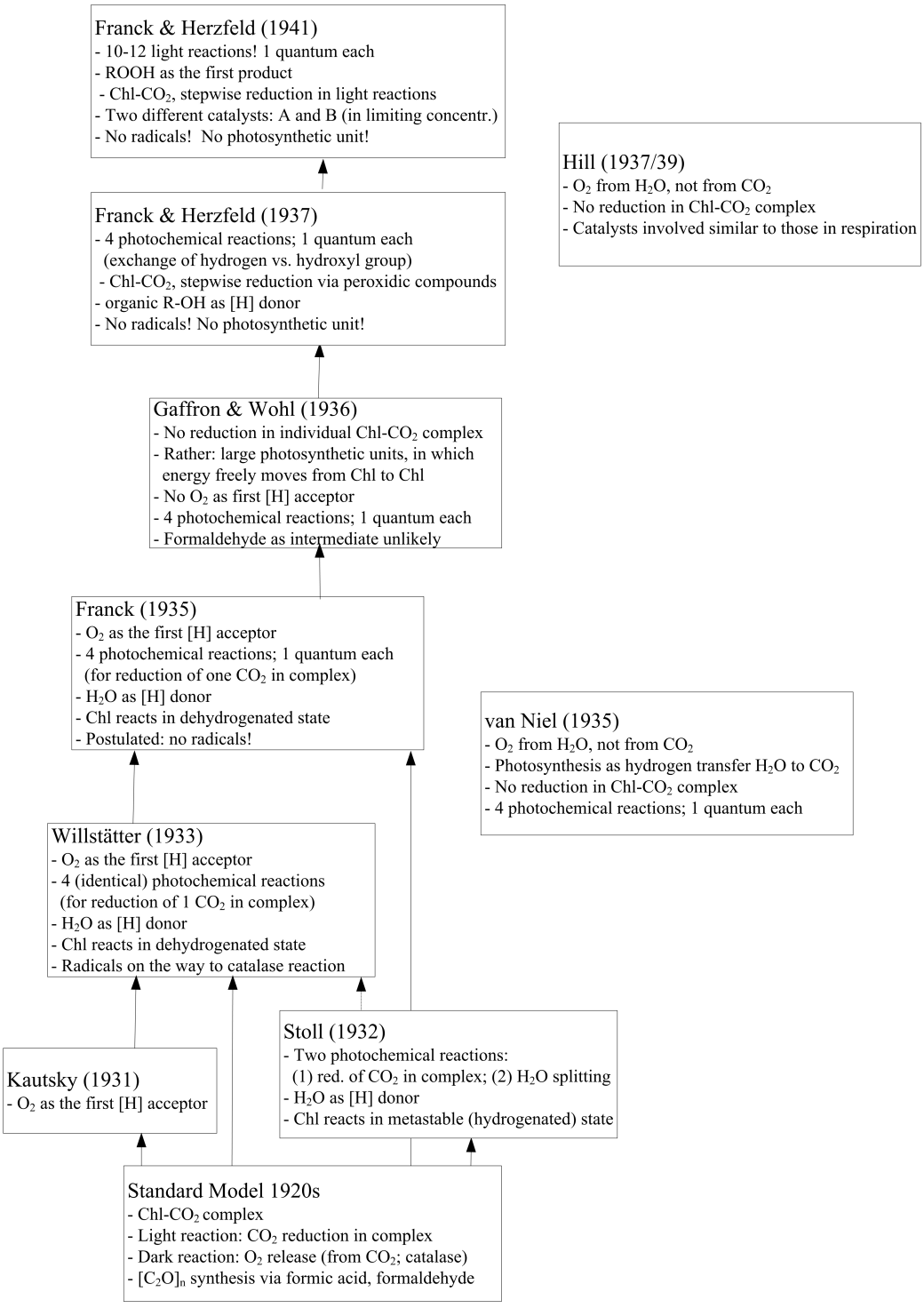

Figure III.10: The important photosynthesis models of the 1930s and their relationships to each other. Only the main characteristics of the models that are not identical to the standard model of the 1920s have been listed.

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 branches of the causal graph – 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: scientists tend to focus on certain partial processes, which means that other processes are consequently going to be ignored.

On the other hand, 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 quantum yield 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 yield of 1. This implied, among other things, that one had to prevent the occurrence 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. III.11, p. 162): having completed some work on the structure of chlorophyll, Stoll took the opportunity to use these findings to contribute to the general

[134] See Haber & Willstätter (1931) for the pertinent publication. The major claim herein was 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). See, on this theory, e.g., Willstätter (1973), pp. 378ff., and Werner & Irmscher (1995), pp. 30–31.

[135] See Werner & Irmscher (1995), pp. 122–123. Letter from Haber to Willstätter, 24 February 1933.

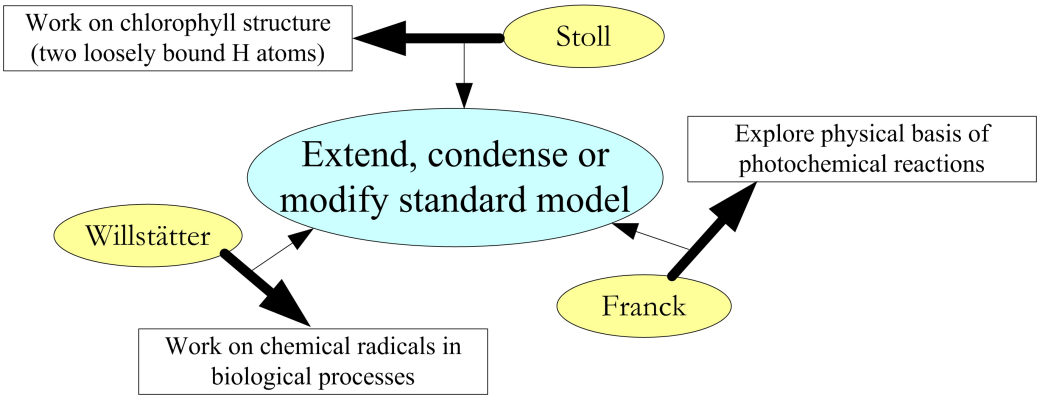

Figure III.11: Actors and their goals: diverging individual (superordinate) goals; extending the standard photosynthesis model as a subgoal or incidental goal. The thick arrows in bold typeface indicate the relationship "X pursues the superordinate goal Y"; thin arrows indicate that, in the course of pursuing the superordinate goal, the incidental goal of contributing to finding the photosynthesis model emerged.

problem of the mechanism; the same holds for Willstätter, although his findings were not concerned with chlorophyll but with the formation of radicals as the central factor of the biological processes that occur in solutions; finally, Franck used his theoretical expertise in fluorescence and energy exchange processes to try and clarify the subject. His approach and 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.

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. It was hardly surprising then that Emerson picked the theme for his first years of independent research after having carefully read the field's classic literature: the photosynthesis monograph by Willstätter and Stoll as well as Warburg's papers. The flashing light experiments were intended to clarify both a phenomenon that Warburg had noted in passing (that one could increase the rate of photosynthesis by using intermittent light and dark periods) and 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 would have been expected. That Emerson hit upon something completely unexpected, which would prove to be the downfall of the standard model of photosynthesis –

as far as the function of chlorophyll was concerned – was, thus, ironic but hardly intentional.

The resulting paper of 1932 presented the surprising findings without, though, giving much of an explanation or too much speculative interpretation. While one could say that Franck played the role of theoretician in the history of photosynthesis research, Emerson was the empiricist; and although there were occasions when Franck acted as an experimentalist and Emerson did, in fact, formulate a theoretical framework, the general tendencies are clear from the papers they presented. 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. However, it is also a matter of education and knowledge. Franck undoubtedly lacked the necessary experimental skills to handle algae and manometers; and Emerson was not a quantum physicist. Finally, however, one should not forget that the same individuals may be able to play different roles in different collectives: Franck started off as an experimentalist in physics, not as a theoretician; it was only in the context of photosynthesis research that he felt he should keep to theory.

The case of the photosynthetic unit nicely illustrates how different background knowledge and context 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, and that this was the factor 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. Emerson and Arnold's finding indicated that somewhere in the process there was a bottleneck; this was identified by Emerson as the enzyme reaction, which, within the standard model, was assumed to follow the photochemical process. Thus, in terms of the underlying causal graph, Emerson argued that the existence of an additional factor should be introduced. Franck, however, shaped his theory with Herzfeld as the inverse of auto-oxidation processes – which 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). Thus, Franck decided to modify slightly the modelling of the course of the photochemical reactions in terms of the intermediates and the mechanism.

Gaffron and Wohl, on the other hand, designed their bold explanatory hypothesis against the background of the Delbrück colloquia, which 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. Equally unsurprising is the fact that this hypothesis was not immediately 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 prove the inaccuracy of the traditional model and to establish firmly the causally relevant factors postulated in their own proposal. Note, however, that Gaffron and Wohl also 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.

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.

It is highly unlikely that van Niel started out with the goal of contributing to photosynthesis research. From all the available evidence, he 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 *Thiorhodaceae* as a test organism should not be overplayed – 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] This is how he stumbled upon the problem of photosynthesis and came across the organism; and although retrospective autobiographies can often be unreliable, this explanation seems entirely plausible.

However, notwithstanding all these contingencies, through his immersion in general microbiology van Niel was, without question, extraordinarily well prepared to conceptualise the metabolism of the group of 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 acceptance of which was necessary if one wished to conclude that there was a generalised equation for photosynthesis, different variants of which were realised in plants and bacteria (they mostly differ in their use of appropriate hydrogen donors). The striking similarity between the possible summary equations

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

of the processes in bacteria and plants was enough to convince van Niel. However, in view of his fragmentary positive evidence, few in the scientific community were ready to accept his conclusion for it was well known that processes which come down to the same summary equation 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 hard, even for microbiologists, to do: it seemed particularly audacious to assume this unity in view of the broad range of reactions observed in bacteria, which adapt so rapidly to changing environments, and the unchanging photosynthesis in plants. [137]

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. The energy required for the process of water splitting would be essentially the same as for the overall photosynthetic reaction, so that the logical consequence was that the reduction of carbon dioxide had to proceed by means of a thermochemical reaction. Accepting these consequences would have made the standard model of photosynthesis untenable. Thus, the neat balance between the volumes of carbon dioxide consumed and the oxygen produced suddenly appeared to be merely coincidental, which was not easy to accept and indeed made the coincidence appear highly unlikely.

Oxygen Evolution in Chloroplasts

It was Hill's work that 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 above 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 an instantiation of a model which explained similar processes under circumstances y (illuminated chloroplasts in plants). Hence, in both cases the heuristic strategy of transferring causal knowledge from one domain to another was put to work; and in both cases the justification of this transference was not immediately successful.

Of course, the assumption that the oxygen produced during photosynthesis came from water and not from carbon dioxide was the most parsimonious choice of theory; and this might well have contributed to the increasing acceptance of the water-splitting concept. It was not that scientists immediately jumped to this

[137] Note that this discrepancy was taken by Franck & 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 secondary reactions and the dehydrogenation of the specific hydrogen donor took place in later stages of the process.

conclusion – for most of the 1940s it was still being debated whether all the oxygen released as a by-product of photosynthesis really did come from water (note that the models proposed by Stoll and Willstätter, for example, at the beginning of the 1930s, had assumed that the photosynthetic oxygen had more than one source). Through the absence of a convincing mechanism to achieve the decomposition of water (which required a very strong reducing agent), a parsimonious hypothesis was only partially convincing.[138]

The Art of Groping in the Dark

In general, the 1930s could be characterised as a period of transition. The decade started with rather stabilising tendencies – the attempts made by Kautsky, Stoll, Willstätter and Franck to make sense of new data by slightly modifying and extending the previous accounts into a new standard model of photosynthesis. However, this was followed by a series of confusing observations, which led to the concepts of a photosynthetic unit and the generalised equation being introduced. Both seemed to indicate that no chlorophyll-carbon dioxide complex was formed, that the oxygen produced during photosynthesis came from water and, finally, that only oxygen formation was a light-driven process, while carbon dioxide reduction was not. It was hard to reconcile all these factors with the standard model.

The reactions were diverse. Experimentalists such as Emerson refrained from making any sweeping theories and confined their interpretations to introducing sofar neglected causally relevant factors (such as assuming that an enzyme at low concentration levels accounted for the peculiar phenomena of the low light saturation point), which, so to speak, was an attempt to amend one branch of the model, notwithstanding the fact that it might have endangered the balance of the whole. On the other hand, more theoretically ambitious scientists, such as Franck, tried their utmost to develop at least tentative visions of an encompassing model that attempted to account for as many sets of data as possible. Both these strategies produced no more than controversial opinions.

However, one common strategy of most of the players was their choice of well-established assumptions as the basis of the rest of their research. For van Niel, it was the vision of biochemical unity: the attempt to express all metabolic processes, which were largely the same in all forms of life, in terms of the transfer of hydrogen. For Franck, on the other hand, it was the extremely high energetic efficiency of the process that crucially determined his models up to 1941. Also, for most other photosynthesis researchers, the quantum yield of the reaction, as measured by Warburg and Negelein in 1923, became an important criterion to which every serious model of the process had to comply. However, the fact that this criterion was extremely hard to meet, soon enough brought several research groups around the same time to re-investigate this parameter; the relief that Franck felt in view of the new measurements of much higher quantum requirements in the late 1930s is tangible when one reads his 1941 paper. The controversy that ensued is examined in the next chapter.

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[138] It was Ruben and Kamen's experiments with "heavy" water, incorporating the oxygen isotope ^{18}O, in 1941 that provided the most convincing evidence for the hypothesis that photosynthetic oxygen comes from water; see Ruben, Randall, Kamen & Hyde (1941) for the publication, which is also discussed in this book; see Chapter V, Section 1.4, p. 266.

A New Concept of Photosynthesis

Another important change in scientists' attitude towards photosynthesis slowly crystallised during the decade looked at in this chapter. It began to dawn on the researchers involved that the process was much more complex than previously imagined. It transpired that many new factors were relevant – for example, the physiological state of the research organism and its developmental history. The growing conditions of the algae (such as exposure to light, pH value, atmospheric pressure, carbon dioxide concentration, and so on) turned out to be highly relevant. Different genera and even species of algae were shown to react quite differently to changes in conditions; even closely related strains of *Chlorella* and *Scenedesmus* algae yielded very different results under equal conditions, so that one would naturally expect dramatic differences between the process of photosynthesis that occurred in organisms as different as algae and higher plants. As soon as processes that were more complex than the transfer of individual hydrogen atoms had been observed, it became clear that the concept of biochemical unity needed to be qualified.

Yet, even when only one organism, grown under the same standard conditions, was investigated, photosynthesis still proved to be a very flexible process. This was addressed most explicitly in Gaffron (1940*b*). Therein, the author pleaded for a move away from the traditional, quasi-mechanical concept of photosynthesis, which (unlike respiration) had always had the same stoichiometry. Gaffron pointed out that 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) only held under stationary conditions, while particularly at the points of transition of, for example, light to darkness, very different ratios were obtained. Gaffron acknowledged that the assumption that the oxygen given off came from carbon dioxide "has been abandoned by most students of photosynthesis"; however, the view that the stoichiometric relations remained the same persisted.[139] From Gaffron's point of view, the interference of photosynthetic processes with many other reactions in the plant was still not sufficiently appreciated. 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. The result would be that none or only a part of the oxygen was liberated while varying amounts of carbon dioxide were formed.

This point was strengthened by Gaffron's finding that, in green algae such as *Chlorella*, a different type of photosynthesis could be artificially induced: namely anoxygenic photosynthesis, which used molecular hydrogen.[140] This closely resembled the form of photosynthesis that occurred with hydrogen in purple bacteria, and this brought Gaffron to endorse strongly van Niel's idea that the mechanism of plant photosynthesis and bacterial photosynthesis might run along principally the same lines. How this new type of anoxygenic photosynthesis differed from the usual process of oxygenic photosynthesis in green algae (that is, how, under certain circumstances, the formation of oxygen was prevented) was unclear. Gaffron raised

[139] Gaffron (1940*b*), p. 204.
[140] See Gaffron (1940*a*).

three possibilities: (1) that hydrogen was able to replace water as the principal hydrogen donor; (2) that hydrogen reduced the intermediate photoperoxides and thus prohibited the release of oxygen; or (3) that the process ran normally, until in the end the oxygen reacted with the hydrogen. Gaffron favoured the second option as the most probable, which also underlined his point that "photosynthesis is not a rigid process with inseparable steps, but a flexible one in which oxygen liberation can be separated from carbon dioxide reduction".[141]

These then were the new uncertainties that faced photosynthesis at the end of the 1930s, even though the surprising developments outlined in this chapter had raised the hope that some major breakthrough was on the horizon. In their review article of 1941, Franck and Gaffron wrote that they still believed that many of the different models of the 1930s appeared to offer possible solutions to the problem, although they added that "as yet there has been little experimental support for the preference of one scheme to another".[142]

[141] Gaffron (1940*a*), p. 282.
[142] Franck & Gaffron (1941), p. 252.

Chapter IV

THE MAXIMUM QUANTUM YIELD CONTROVERSY (1937–55)

1 Introduction

Measuring the quantum yields and quantum requirements of photochemical reactions first became standard practice in the fields of physics and photochemistry during the first decade of the twentieth century.[1] These measurements concerned the efficiency of a photochemical process; or, to put it in quantitative terms, they either showed how many light quanta were required to yield one molecule of product (this was the quantum requirement) or how many product molecules were released through the effect of one light quantum (this was the quantum yield, which is the reverse of the quantum requirement). The epistemological value of these parameters was enormous because once it was known how much energy in terms of light quanta a process required, then it became much easier to reconstruct the process: many of the possible pathways looked far less attractive than before when it was found that they did not fit the determined energy budget; and for some of them appropriate modifications were not easy to be found. However, for many years few biologists paid any attention to taking quantum measurements; questions like these were confined to the submicroscopic world of theoretical physics, and most biologists seemed to consider them irrelevant.

It was the physiologist and biochemist Otto Warburg who, thanks to the work of his father Emil, had become familiar with the practice of taking quantum yield measurements and introduced it to photosynthesis research (see Chapter II of this book). Together with Erwin Negelein, Warburg had found that a minimum value of four to five quanta of light was required to produce one molecule of photosynthetic oxygen (while one molecule of carbon dioxide was consumed).[2] The important qualification here is the adjective "minimum" – for the quantum requirement of photosynthesis was found to be not a constant value but a function of a whole range of parameters, which included light intensity. Yet, only the lowest number of quanta required was of theoretical importance: according to Einstein's Law of Photochemical Equivalence only then could one draw conclusions as to the number of photochemical steps involved and to the amount of energy necessary to make the process operate.

As mentioned earlier, the quantum yield of photosynthesis was regarded by most of the photosynthesis experts working in the 1930s as a vital piece of in-

[1] This chapter was written in collaboration with Govindjee of the University of Illinois, Urbana–Champaign, Illinois (United States), to whom I extend my thanks. Govindjee generously shared substantial pieces of information, which proved crucial for writing this chapter, with the author. He also kindly discussed with me the issue of respiration's interference in the process of photosynthesis, which proved to be the central confounding factor in the debate (and pointed out its specific relevance); and he drew my attention to the importance of the choice of buffer solution. A lengthened and differently focused version of the content of this chapter is to be published in a study co-authored with Govindjee; earlier contributions to this topic written by Govindjee include Govindjee (1999) and Govindjee (2004*b*).

[2] Warburg & Negelein (1923).

formation (see Chapter III). The 1923 Warburg–Negelein value of four to five quanta was generally accepted, and it remained virtually unchallenged until around 1937. Not only did the experiments appear well-founded and the results conclusive – which was hardly surprising, considering that Warburg was renowned for his exceptional skill in conducting experiments (one had to be extremely meticulous when measuring the quantum yields of life processes) – but the value of around four also nicely matched theoretical expectations: the conversion of water and carbon dioxide into molecular oxygen and a moiety of carbohydrates required a minimum calculated energy input of 112 kilocalories (kcal), while red light, which was known to be the most efficient region in the spectrum for bringing about photosynthesis, carried about 40 kcal per molecule of light quanta. Thus, at 100 per cent efficiency of the process, photosynthesis would require 2.8 light quanta per oxygen molecule; and since no process could possibly run at total efficiency, a value slightly higher than this was to be expected. The value of four, furthermore, seemed to be highly significant to many photosynthesis researchers, because it corresponded so neatly to the four hydrogen atoms (or, alternatively, electrons) that had to change their places and bondings in the process of turning CO_2 into $[CH_2O]$. Yet, however well all this fitted together, at least on paper, a quantum requirement of four to five was still so close to the theoretical limit given by the calculation above that it was difficult to devise a model of the mechanism that could convincingly explain the relevant empirical findings.

Thus, in view of the value's importance, it is not surprising that in the second half of the 1930s several research groups started to re-examine the question independently, which quickly resulted in serious concerns being raised about the standard value's validity. Warburg and Negelein's findings were increasingly questioned – most vigorously by Robert Emerson, together with several of his co-workers, who harshly criticised the methods that Warburg and Negelein had applied. In line with some other teams working in the United States (US), Emerson argued that eight to twelve light quanta were needed for photosynthesis. Papers and arguments were exchanged, but no agreement reached, which led Emerson to invite Warburg to his laboratory at the University of Illinois at Urbana–Champaign in 1948, the idea being that the two researchers would compare their experimental protocols and thereby settle the disconcerting discrepancies that had arisen – disconcerting, because both Emerson and Warburg were renowned for their mastery of manometry. Both used the same technique and the same organism – indeed, as mentioned in Chapter III, Emerson had been trained by Warburg; yet they obtained data that differed by a factor of two or three. However, Warburg's stay in Urbana proved unfruitful: nothing was settled and the two opponents parted as enemies. Consequently, the controversy continued to grow during the 1950s – to such an extent that the biophysicist Roderick Clayton commented, in 1965, that "the quantum efficiency of photosynthesis became perhaps the most exhaustively measured phenomenon in the history of science". It was only in the 1960s, when models that included two different light reactions became the norm in photosynthesis, that the issue was considered settled in favour of the higher quantum requirement, although Warburg never accepted this solution.

The general story of this controversy, as outlined above, has been told many times in photosynthesis research circles.[3] However, there are certain turns and details that only come to the fore in an analysis based on archival sources, which up to now has never been comprehensively undertaken. Using the episode as a starting point, I shall examine the controversy's place in the history of modelling photosynthesis, and explain why this controversy, which lasted for more than twenty years, turned from being a constructive exchange of diverging opinions (at least on the part of Emerson) into a dead end, which essentially impeded Warburg and Emerson from making more fruitful contributions to the field.

2 First Opponents of the Warburg–Negelein Value

There were a number of researchers who, during the course of the 1930s, opposed the Warburg–Negelein value of the maximum quantum yield, the first being William A. Arnold (the same Arnold who in 1932 had collaborated with Emerson in the flashing light experiments) in his 1935 doctoral thesis. Arnold had followed Emerson's recommendation and moved from the California Institute of Technology (Caltech) to Harvard University (Cambridge, Massachusetts), where he entered the graduate programme in General Physiology. Part of his project was to measure the minimum quantum requirement of photosynthetic oxygen, and in order to carry this out Arnold developed microcalorimetric techniques, which were quite different from manometry. (In microcalorimetry, the process is not monitored by registering pressure changes but by determining the resulting heat flow in a leaf or a cell suspension.) By this means, Arnold found that a minimum number of eight light quanta were required to produce one molecule of oxygen during photosynthesis.[4] Although the number eight was two times greater than the Warburg–Negelein value of four, it seems that Arnold assumed at the time that eight was low enough to be included in the same range. This would explain why he not only refrained from immediately crying out his results but also did not have them published.[5]

Arnold's results were published much later, in Arnold (1949), and, as he claimed in this paper, only at the insistence of Hans Gaffron, who had assured Arnold that his quantum yield values had become important in view of the developing controversy on the subject.[6] Yet, Arnold did not even mention that the maximum quantum yield that he had measured differed from that of Warburg and Negelein

[3] See, e.g., the comprehensive collection of historical perspectives, tributes, etc., in Govindjee et al. (2005), all of which include an array of further references.

[4] Arnold (1935, pp. 35–38, Table IX; PhD thesis at Harvard University, Cambridge, Massachussetts) reported, in *Vallisneria*, an efficiency of 35% (equivalent to a minimum number of eight photons/O_2) in red light of 420 ergs/cm^2/s, at a temperature of 22°C.

[5] Arnold later continued his microcalorimetric measurements in Cornelis van Niel's laboratory at the Hopkins Marine Station in California. This work of Arnold's, carried out in 1938, was recognised in a footnote in Magee, de Witt, Smith & Daniels (1939).

[6] Arnold's results, which he obtained using *Chlorella*, showed that the minimum number of quanta per O_2 evolved, calculated from microcalorimetry measurements, was never lower than 9 per molecule of CO_2 absorbed (see Table 13.1 in Arnold (1949), p. 275). In the first sentences of the paper, Arnold himself admitted that his earlier findings were being published at Gaffron's insistence: "This study was carried out at the University of California and the Hopkins Marine Station during the years 1936 and 1937. It is being published because of the urging of Dr. Gaffron and because Warburg has re-opened the question of quantum yield in photosynthesis." (p. 273)

– Arnold still believed that he had confirmed Warburg and Negelein's finding of an extremely low quantum requirement, so that there was no need to discuss the question any further.[7] This was characteristic of Arnold's general attitude to putting his results into print: although Arnold contributed greatly to photosynthesis research as well as to many other scientific areas, he was always reluctant to publish his findings. Indeed, Arnold is remembered for having said that scientific results should be engraved in stone: he believed that, faced with such a formidable task, scientists would be much more discerning about what was publicised, and that publications would, as a result, become more worthy.[8]

Around the same time, an interdisciplinary research team at the University of Wisconsin in Madison also started to re-evaluate the quantum yield requirement. This team included the plant physiologists Winston M. Manning, J. F. Stauffer and Benjamin M. Duggar as well as the eminent photochemist Farrington Daniels. In 1938, they presented the first published challenge to Warburg and Negelein's quantum yield value.[9] For their experiments Manning et al. had developed a (rather cumbersome) chemical gas analysis method, which they applied to *Chlorella* cells. By this means, they had arrived at a minimum quantum requirement of sixteen to twenty quanta per molecule of oxygen evolved – diverging from the standard value by a factor of four or five. With hindsight it is clear that many of their experiments were not in the range where minimum quantum requirement ought to be measured (namely, at very low light intensities). However, this publication caused quite a stir in the photosynthesis research collective, which was reinforced when a year later the group published new results (taken using microcalorimetric measuring techniques), showing that twelve light quanta were required to produce one molecule of oxygen, or, respectively, consume one molecule of carbon dioxide.[10]

Quantum yields were also measured in the late 1930s by the physicist Foster F. Rieke, who at the time was James Franck's assistant at Johns Hopkins University in Baltimore (Maryland).[11] Given the importance that Franck attached to the quantum yield in his papers (discussed in Chapter III), it is not surprising that Franck encouraged his assistant to investigate the value again. Rieke explicitly stated in his paper that the results of the group based at Madison as well as other "unpublished reports", as Rieke put it, which had also failed to reproduce the Warburg–Negelein value, had prompted him to undertake this research.[12] Rieke had decided to use Warburg's own manometric techniques in order to discover whether he could duplicate the original findings. In this he succeeded, as he arrived at an average value of about five quanta for the minimum requirement: a fair confirmation of Warburg–Negelein. However, Rieke found that the values strangely varied according to the method of calculation, which led him to conclude that

[7] Arnold (1949). The dry summary of his findings reads: "In no case did the number of quanta used per CO_2 molecule reduced fall below nine." (p. 276)

[8] Cf. Govindjee, Knox & Amesz (1996*b*), p. 1; Govindjee, Allen & Beatty (2004), p. 4.

[9] Manning, Stauffer, Duggar & Daniels (1938).

[10] See Magee et al. (1939). Out of the seventeen experiments that they conducted, ten gave a value of approximately ten quanta per oxygen evolved. The authors clearly recognised that some of the earlier experiments with higher values were incorrect.

[11] Rieke (1939). See Guttman, Hess, Myers & Wolfe (1970) for Rieke's obituary.

[12] Rieke (1939), p. 238.

"either there is an obscure systematic error in one method of measurement or, under the conditions of the experiments, photosynthesis and respiration do not follow a simple course".[13] It is worth pointing out, for reasons that will be clarified later, that Rieke obtained his lowest figures only when he used a phosphate-containing medium, following the recipe of Dean Burk, a biochemist who was also working at Johns Hopkins at the time and whose help was acknowledged in Rieke's paper (and who had become famous thanks to his 1934 paper co-authored with the American physical chemist Hans Lineweaver).[14] On only one occasion did Rieke use a carbonate-bicarbonate buffer solution, in which he measured a minimum requirement of about eight quanta: "The quantum efficiency was reduced 40 per cent," Rieke stated in view of this aberrant run, while he surmised that some property of the buffer solution was responsible for this diverging value.[15]

From Rieke's correspondence with Franck at the time, one can see that the latter found these values satisfactory. On 29 July 1938, Franck wrote to his assistant:

> I agree entirely to each remark you make and I am also very content with the result that the quantum yield is between 1/4.5 and 1/5. I think there is no sense in going on further and I am pretty convinced that the publication of the results will find great interest among the people working in that field.[16]

Later correspondence shows, however, that Rieke nevertheless intended to continue with his studies. In his 1939 paper, for instance, he pointed to the fact that it would be desirable "that methods be introduced which avoid as far as possible a large correction for respiration, and such experiments are being undertaken by the author".[17] Yet, in July 1939, Rieke admitted in a letter to Franck that he had not been as productive as expected: "I must report that I have made no progress in improving the quantum efficiencies." This was to remain the state of affairs for the next ten years, even though Franck continued to ask after Rieke's work. In 1942, Franck even turned to Mrs Rieke for help in securing the paper, pleading that it did not matter if it was not entirely finished: "If only the actual results are in it, we can add some 'gravy and potatoes' to the 'main course' and send it back to you, and if Foster doesn't like our additions, he may throw them out." To which Franck added: "I think we just have to publish that paper soon; otherwise, Foster's whole efforts throughout several years may be in vain."[18] Nonetheless, despite a number of ever more pressing requests from Franck, it was only in 1949 that Rieke's data of the years 1939-41 saw the light of day. (Several factors may have contributed to the delay in publication; in addition to other distractions, Rieke's involvement in war-time work with the Magnetron Group of the Massachusetts

[13] Rieke (1939), p. 243.

[14] Cf. Lineweaver & Burk (1934), in which the famous Lineweaver–Burk plot (or double reciprocal plot), which became so useful in enzyme studies, was introduced. This work is apparently the most frequently cited paper ever published in the *Journal of the American Chemical Society*.

[15] Rieke (1939), p. 243. See Table IV. p. 242.

[16] Franck to Rieke on 29 July 1938. Franck, James. Papers, [Box 7, Folder 9], Special Collections Research Center, University of Chicago Library

[17] Rieke (1939), p. 239.

[18] Franck to Mrs Rieke on 9 May 1942. Franck, James. Papers, [Box 7, Folder 9], Special Collections Research Center, University of Chicago Library.

Institute of Technology (MIT) Radiation Laboratory in Cambridge, was most probably a cause.[19]) The result of Rieke's prolonged efforts was that, according to his experiments, "a preponderance of evidence indicates that the maximum efficiency lies between the limits 0.09 and 0.11, and that there is no unequivocal evidence that it is appreciably greater than 0.12".[20] Or, put the other way round, the minimum quantum requirement for photosynthesis as measured by Rieke was nine to twelve.

In the meantime, another major protagonist had entered the stage. Already in his 1939 paper, Rieke had acknowledged that, in a lecture given at a symposium on photochemistry at Stanford University (Palo Alto, California) in August 1938, Emerson had criticised the methods used by Warburg and Negelein. On 20 July 1939, Emerson turned directly to Rieke, in response to the latter's paper of April of that year, in which the value of five had been published. Emerson wrote that his group had arrived at the same low quantum requirement values as Rieke, but only when they had used a very specific medium for the cells:

> When we tried to repeat them [the experiments] using the medium which you specified, we were unsuccessful. Thinking that you had probably followed Warburg & Negelein in using tap water, we sent to Baltimore for some tap water, and with this we were at once able to duplicate your results.

And then Emerson added the following lines, which included the stunning announcement that he had obtained a quantum yield of three, which would have implied an efficiency of almost 100 per cent:

> We are preparing some of our results for publication, and including a description of a medium in glass-distilled water in which we can regularly produce cells giving *quantum yields of about 3 quanta per* CO_2. We think it should be easy to duplicate these results in other laboratories by following a more standardizing technique. If you care to try out our medium, I shall be glad to send you a full description of it in advance of publication.[21]

Rieke's subsequent letter to Franck shows that he, too, had struggled with the culture medium: "Judging from the letter I am enclosing, Emerson has more than solved the problem of how to grow the algae." He then inquired what Franck thought about a quantum efficiency of three, which Rieke himself found "very difficult to believe".[22] Rieke subsequently tried to reproduce Emerson's findings, yet constantly failed (he always arrived at values of nine to ten); so that finally Rieke went to Palo Alto in California to see Emerson (who was then on leave of absence from Caltech and working at the Carnegie Institution of Washington at Stanford University) and discuss the matter with him in person.

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[19] See footnote 1 in Rieke (1949); see also the MIT Radiation Laboratory Oral History Project, carried out in 1991 under the auspices of the IEEE (Institute of Electrical and Electronics Engineers) Global History Network, in which Rieke's contributions to the MIT Radiation Laboratory are mentioned by several participants. The interviews can be accessed at <http://www.ieeeghn.org/wiki/index.php/MIT_Radiation_Laboratory_Oral_History_Project>.

[20] Rieke (1949), p. 270.

[21] Emerson to Rieke on 20 July 1939. Franck, James. Papers, [Box 7, Folder 9], Special Collections Research Center, University of Chicago Library.

[22] Rieke to Franck on 25 July 1939. Franck, James. Papers, [Box 7, Folder 9], Special Collections Research Center, University of Chicago Library.

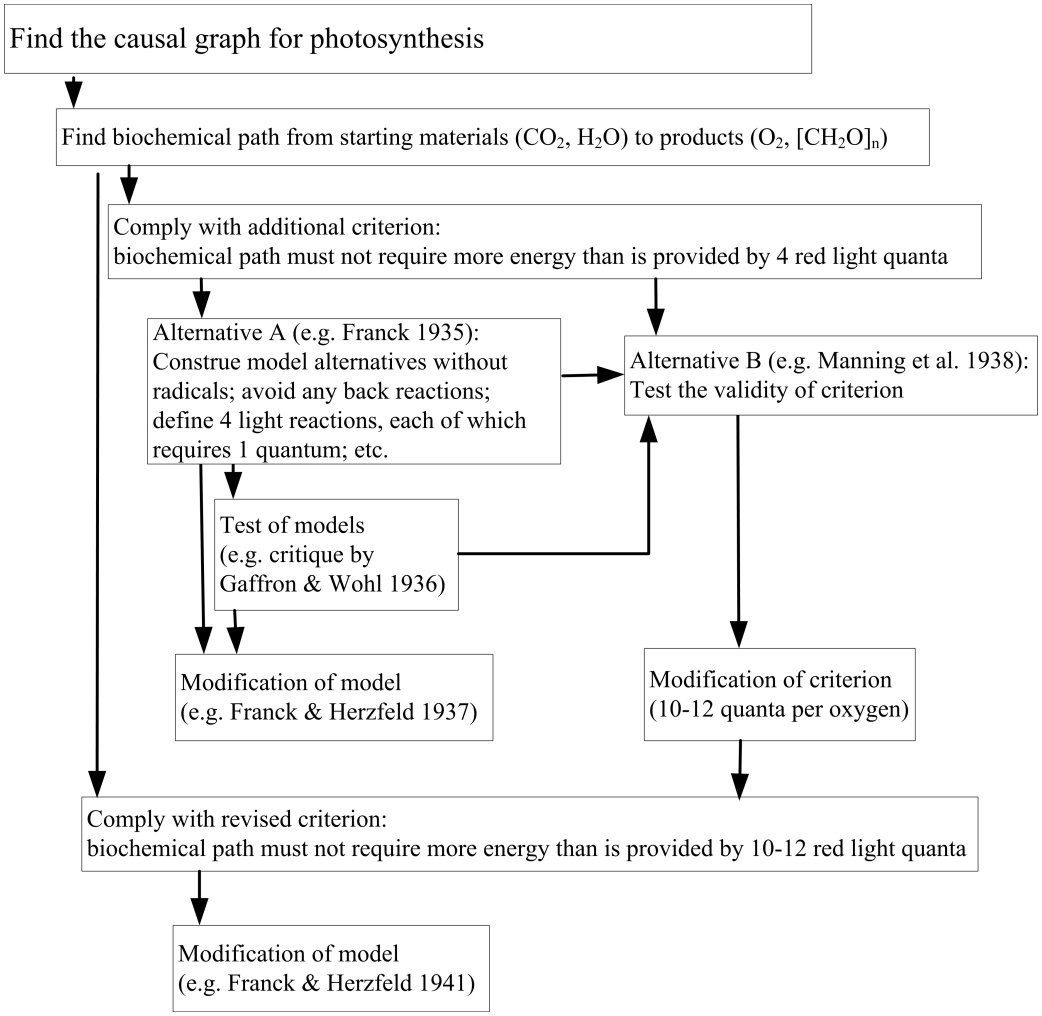

Figure IV.1: The testing of the validity of a photosynthesis quantum yield of four quanta per molecule of oxygen emerged as a new high-priority goal. Acceptance of the new criterion (an energy budget of ten to twelve quanta) led to a new family of models being formulated, among those that of Franck & Herzfeld 1941.

It is clear that, by the end of the 1930s, the theme of photosynthesis efficiency was being vigorously debated in a number of laboratories – at least in the US. The reason for this is not hard to find. As was shown in the last chapter, the quantum yield of photosynthesis provided one of the very few criteria that models had to meet in order to be acceptable. The Warburg–Negelein value of four quanta per molecule of oxygen had gone unquestioned for more than ten years, even though the value was too close to the thermodynamic maximum. Yet because of the difficulties researchers encountered when they tried, in the 1930s, to make the models comply to this tight energetic constraint, it seemed worthwhile to re-investigate the figure, if only to confirm the criterion. The revised figure of ten to twelve quanta per molecule of oxygen opened the way for a new family of models, one of which was the one presented in Franck & Gaffron (1941). (See also fig. IV.1, p. 175.) I shall now turn to the work of Emerson in more detail, as he quickly became the most vigorous opponent of the Warburg–Negelein value.

3 Emerson and Lewis's Challenge

It is not entirely clear at which point Emerson began to doubt the validity of the Warburg–Negelein experiments. Although he was still convinced of Warburg's efficiency values in 1932, five years later, in 1937, Emerson embarked on an extended project to revisit the quantum yield question. He took a leave of absence from Caltech, and spent two years at the Plant Biology Laboratory of the Carnegie Institution of Washington (on the campus of Stanford University). Emerson was the guest of Herman A. Spoehr, author of one of the first monographs on photosynthesis, and he was also able to benefit from working with the skilled physicist C. Charlton M. Lewis.[23] On 5 November 1938 Emerson wrote a letter to Warburg describing the project. Among other things, Emerson reported to Warburg the findings that Lewis and he had arrived at so far; for example, they had encountered relatively large induction effects, which lasted no less than five minutes after the onset of illumination:

> We tried everything to make these induction effects disappear, we chose a faster shaking velocity, we provided for better mixed suspensions by having a little glass spoon fitted to the lid of the vessel, etc., but we are convinced that this induction is of a biological, not a physical nature. It also changes in an interesting way, depending on the duration of the previous dark period. I think that if one abruptly illuminates the cells, first only oxygen is formed, so that the vessel constant is incorrect for the moment, until the ratio CO_2/O_2 turns back to normal. This may happen only in the following dark period.[24]

Emerson also reported that he had no trouble arriving at a value of five quanta per molecule of oxygen, but had so far not succeeded in measuring a value of four. This is remarkable, since, in contrast to Arnold, who had thought that eight to ten was in the same range as four to five, Emerson seemed to have considered this difference *between four and five* significant. At the same time, Emerson already felt

[23] See Govindjee & Krogmann (2004), p. 47, for a short obituary of Lewis.

[24] Archive of the Berlin-Brandenburg Academy of Sciences and Humanities (Archive of the BBAW), NL Warburg 262. Emerson to Warburg, 5 Nov. 1938.

that there might be more problems than Warburg and Negelein had acknowledged. In addition to the type of water used, discussed with Rieke, and the induction period, mentioned in the quotation above, Emerson found that, at the change from light to dark and *vice versa*, the gas exchanges had strong oscillations, which he was not yet able to explain. Furthermore, he was concerned about the handling of the bolometer. Emerson believed that Warburg might not have operated it correctly: bolometers were usually set up in a horizontal position, but Warburg and Negelein had placed them vertically, which implied that they risked having a measuring error of about 10 per cent (which for Emerson was important). At the time of the letter, Emerson did not yet foresee the enormous consequences of his findings; but, one year later, in December 1939, Emerson was adamant that the values reported by Warburg and Negelein were incorrect. On a Christmas card to Warburg, he wrote:

> I shall send you a reprint in January, because I believe you will be interested in our results. The [maximum quantum] yield really is not as high as you and Negelein thought.[25]

3.1 The Carbon Dioxide Burst (1939-41)

The article Emerson had alluded to in his Christmas card was published as Emerson & Lewis (1939) and entitled "Factors Influencing the Efficiency of Photosynthesis". In this paper, the authors systematically explored the external factors that influence the photosynthetic yield. It was demonstrated that this value was strongly dependent not only on the type of water used but also on the addition of certain heavy metals, the culture's exposure to light, the age of the culture and the wavelength of light at which it had been grown. Keeping the cultures at lower temperatures also tended to increase photosynthetic efficiency. Thus, the whole issue transpired to be far more complicated than had previously been believed. As a great many factors were involved and they also appeared to be closely intertwined, Emerson and Lewis admitted that this made a causal analysis extremely difficult:

> In seeking to identify the culture conditions which determine the efficiency of the photosynthetic apparatus, we have accumulated evidence that optimal adjustment of any one factor depends somewhat on other factors. We have tried as far as possible to combine the factors in such a way as to obtain the highest possible efficiency, but it has been necessary to make arbitrary choices in regard to certain factors, in order to study the influence of others. Further attention to the interdependence of the various factors may be expected to lead to higher values for the photosynthetic efficiency.[26]

However, in conditions that they suspected to be optimal, and otherwise following Warburg's experimental protocol, Emerson and Lewis arrived at the surprising yield of 0.33 molecules of carbon dioxide assimilated per absorbed light quantum – that is, a quantum requirement of no more than three! (This was the value that Emerson had indicated to Rieke in his letter of 1938.) However, Emerson and Lewis did not believe that this value, which was beyond all theoretical expectations, really

[25] Archive of the BBAW, NL Warburg 262. Emerson to Warburg, Dec. (Christmas) 1939.
[26] Emerson & Lewis (1939), p. 812.

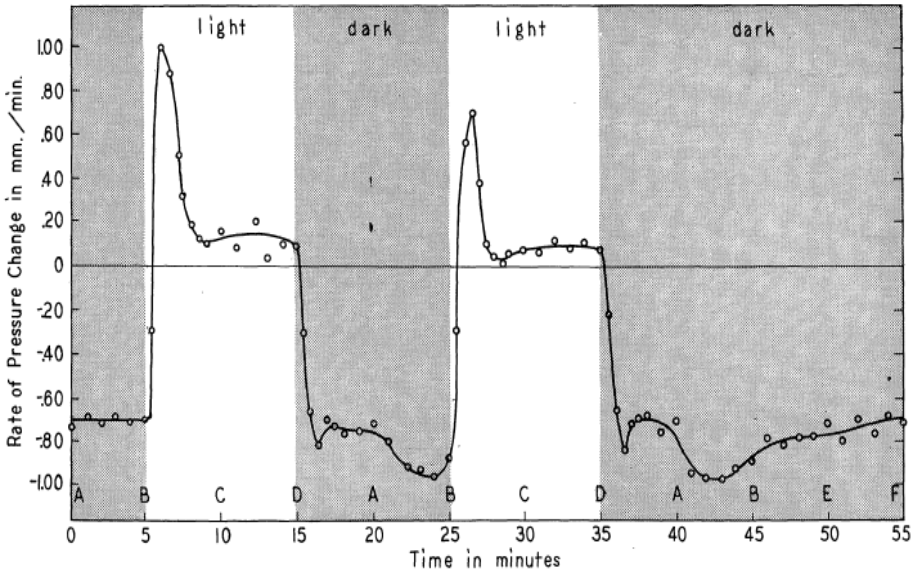

Figure IV.2: Reproduced from Emerson & Lewis (1939), p. 815. Deviations observed in the rate of pressure change during successive periods of light and darkness. The shaded areas represent the dark periods. Light intensity: 1400 ergs/cm^2/sec.

reflected the efficiency of photosynthesis. Emerson and Lewis rather suggested that these exceedingly high values were an artefact of the technique. The decisive source of error was identified as being the curious gas exchange effects that appeared whenever the light source was turned on or off. (Emerson and Lewis also discussed the problem of respiration changes from dark to light but decided that this factor could not account for the magnitude of error involved.) These were the very effects that Emerson had mentioned in his 1938 letter to Warburg (see quote on p. 176). In their paper, Emerson and Lewis wrote:

> With our improved technique we found that after a change from light to dark, or *vice versa*, the rate of pressure change was subject to large deviations before coming to the new steady value. [...] When the light is turned on, a sharp increase of pressure occurs at once and lasts from two to five minutes. Under some circumstances the maximum rate attained may be two or three times the steady rate in the light (the respiration correction being included in each case). [...] When the light is turned off, the rate of pressure change returns approximately to its former (negative) value for a few minutes but then shows an increase. The maximum is reached in about seven minutes and may be 20

> per cent above the steady rate, which is regained after 10 to 20 minutes. [See also fig. IV.2, 178.][27]

Emerson and Lewis were persuaded that these deviations were due to changes in the ratio between oxygen and carbon dioxide; in fact, the sudden peak after the onset of illumination was shown to be mainly caused by the evolution of carbon dioxide. The authors concluded, rather succinctly: "This implies that for the short periods of darkness and illumination used for efficiency measurements the assumptions on which photosynthesis is computed from pressure changes become incorrect."[28]

This last sentence effectively dismissed all previous quantum yield determinations, all of which had depended on the premise that the ratio CO_2/O_2 during photosynthesis, denoted by γ, was unity. Warburg and Negelein had attempted to double-check this assumption, and found the ratio to be -0.9; yet they had arrived at this value at high light intensities and from measurements carried out over a period of time of more than one hour.[29] Emerson and Lewis strongly suspected that the value obtained under these conditions would not be the same at low light intensities and during shorter periods of illumination – that is, for the conditions used in the actual quantum yield experiments. Thus, Emerson and Lewis wrote:

> In view of the apparent variability of γ disclosed by our results, measurements made in this way cannot be accepted as significant until the method has been applied in such a way as to permit the simultaneous determination of both carbon dioxide and oxygen exchange.[30]

Two years later this two-vessel method, which enabled the exchanges of oxygen and carbon dioxide to be measured simultaneously, was presented in Emerson & Lewis (1941*a*). The trick was to use two vessels containing the same quantity of identical algal suspensions, which, however, had different gas-to-liquid ratios (see fig. IV.3, p. 180). This enabled the researchers to calculate the exchange of carbon dioxide and the exchange of oxygen independently of each other. By this means, Emerson and Lewis were now in a position to trace the development of γ in quantum yield measurements, without simply assuming its constancy. And their findings fully justified the tentative objections raised in 1939: the value of γ was extremely unstable, with the greatest variation taking place in the first ten minutes of light or darkness (that is, in the exact time slot that Warburg and Negelein had used for their readings). Emerson and Lewis concluded that this was the cause of a considerable systematic error in the Warburg–Negelein values. To measure the rate of photosynthesis, Warburg and Negelein had chosen the first five minutes of a light period, which included a sudden and significant *increase* in pressure, which was not due to a rise in photosynthetic oxygen, while to measure the rate of respiration, which they used as their correction factor, Warburg and Negelein had chosen the first five minutes of a dark period, which comprised a significant *decrease* in pressure. Together, these time slots led to the efficiency of photosynthesis being greatly overestimated.

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[27] Emerson & Lewis (1939), pp. 814–815.
[28] Emerson & Lewis (1939), p . 815.
[29] Cf. Warburg & Negelein (1922) and Warburg & Negelein (1923).
[30] Emerson & Lewis (1939), p. 817.

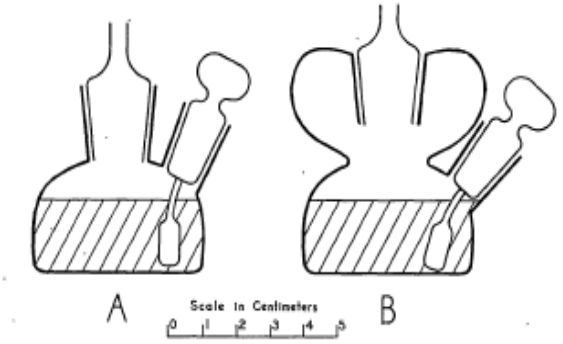

Figure IV.3: "Diagrammatic cross sections of the two shapes of manometer vessels used for determination of oxygen and carbon dioxide exchange. The space occupied by fluid is nearly the same shape and volume in each vessel, but the gas space is much larger in vessel B. The vessels are circular in plan, diameter 5 cm." Reproduced from Emerson & Lewis (1941a), p. 790.

Emerson and Lewis demonstrated that the effect was mainly due to dramatic changes in carbon dioxide pressure, while the oxygen pressure gave a relatively steady course of values: "It is as if the cells contained some sort of reservoir which pours out carbon dioxide in the first minutes of illumination, and which must be filled again in the dark before the full respiration rate of carbon dioxide production can manifest itself."[31] (This phenomenon was shortly afterwards termed the "carbon dioxide burst".) Thus, Emerson and Lewis suggested that computing the rate of photosynthesis and its quantum yield from oxygen changes alone might take care of the problem. By this means, the authors arrived at values of about 0.10 molecules of carbon dioxide per absorbed quantum of light, under widely varying conditions and using no fewer than eleven different species of algae. This value, a minimum quantum requirement of ten, Emerson and Lewis emphasised, was in satisfactory agreement with the values reported by Manning et al. (1938), although they had arrived at it using very different methods. Emerson and Lewis were convinced that this was a fair approximation of the actual value, and considered the issue to be settled.

3.2 The Red Drop (1943)

When Emerson and Lewis set out to investigate this value and related questions a little further, they hit upon a phenomenon that was to have lasting significance. The subject of their paper Emerson & Lewis (1943) was the relationship between the photosynthetic quantum yield and the wavelength of light; and it was in this paper that they published for the first time what would later become known as the "Red Drop" of photosynthetic efficiency.

[31] Emerson & Lewis (1941*a*), p. 794.

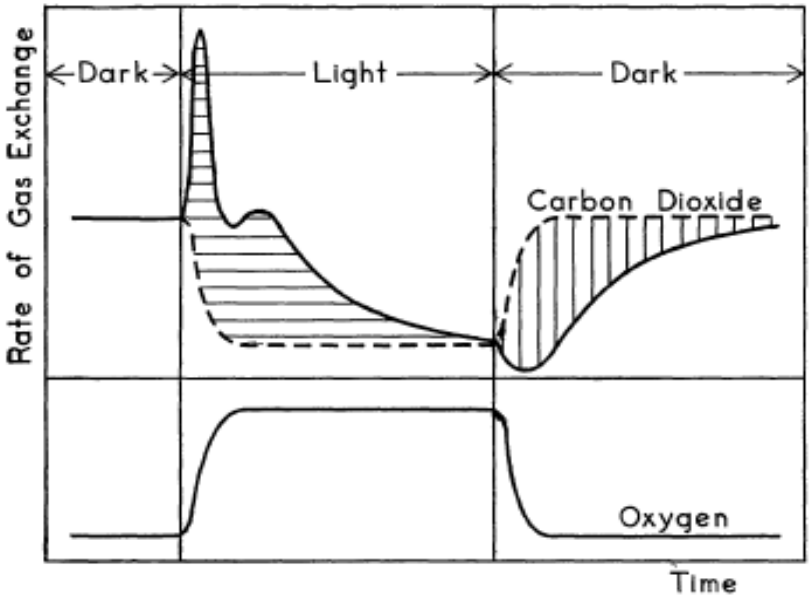

Figure IV.4: "Diagram to show the part of the carbon dioxide exchange attributed to some process other than respiration and photosynthesis. The observed rates of carbon dioxide and oxygen exchange are drawn as solid curves above and below the zero line respectively. [...] The broken curve is drawn on the assumption that the respiratory and photosynthetic quotients are unity and shows the course of carbon dioxide exchange due to respiration and photosynthesis. The shaded area shows the difference between the assumed carbon dioxide exchange and the observed exchange. Horizontal shading is used for areas above the dotted curve, indicating carbon dioxide production in excess of that due to respiration and photosynthesis. Vertical shading is used for areas below the dotted curve, indicating a deficit in the expected carbon dioxide production for respiration." Reproduced from Emerson & Lewis (1941a), p. 794.

The authors started off with the observation that, from a theoretical point of view and given Einstein's Law of Photochemical Equivalence, the efficiency of photosynthesis should be independent of the wavelength, at least for the range of the spectrum in which chlorophyll absorption was high. (This was due to the assumption that the primary photochemical process was proportional only to the number of absorbed quanta, irrespective of their wavelength.) Warburg and Negelein's 1923 measurements had been received up to then as demonstrating exactly this independence; however, since Emerson and Lewis considered that the work done by Warburg and Negelein was methodologically flawed, they saw a definite need for more precise information, "particularly in the red region where chlorophyll is the principal light-absorbing pigment".[32] Emerson and Lewis found the following:

> In the course of the experimental work, two phenomena were encountered which raised special problems in the measurement of the quantum yield in certain portions of the spectrum. Exposure of cells to the blue-green region sometimes caused a considerable increase in the apparent rate of respiration. An unexpectedly sharp decline in the quantum yield was observed in the far red. Since these two observations are probably at least as significant as the original purposes of the work, they are discussed more fully than might appear to be necessary in view of their apparent treatment merely as difficulties of an experimental character.[33]

The first of these findings again undermined one of the previously held fundamental methodical assumptions: up to this point all photosynthesis measurements taken manometrically had used the same respiration correction factor obtained in the dark for all conditions; now, Emerson and Lewis had found that the effect of light-induced oxygen consumption (whether this was respiration in the strict sense or not) could vary significantly at certain wavelengths of blue light around 480 millimicron (mμ).[34] Not all cells were equally sensitive to this wavelength region; however, the effect was always recognisable, so that one should be aware, the authors underlined, of the possibility of variation under different conditions, which might introduce yet another source of systematic error to the photosynthesis rates measured. For example, Emerson and Lewis reported that "under the conditions of the quantum yield measurements, the rate of respiration tends to decline slowly over a period of several hours", regardless of the kind of illumination or other parameters, while the rate of decline was not the same during continuous light or alternating light and dark periods.[35]

Second, Emerson and Lewis found that the quantum yield was roughly constant in the region 580 to 685 mμ, whereas from 685 mμ towards the infrared region of

[32] Emerson & Lewis (1943), p. 165.

[33] Emerson & Lewis (1943), p. 166.

[34] Today the nanometre (nm) is more commonly used than the millimicron (mμ). Nevertheless, the latter was used here in order to keep the main body of the text consistent with the quotes. One nanometre (and one millimicron) equals ten Ångström, another unit formerly used for very small lengths.

[35] Emerson & Lewis (1943), pp. 169–170. Notwithstanding these findings, the assumption that by using ten-minute intervals the rate of respiration in the dark approximated its rate in the light remained part of the body of standard knowledge for a surprisingly long time, although it was clearly wrong.

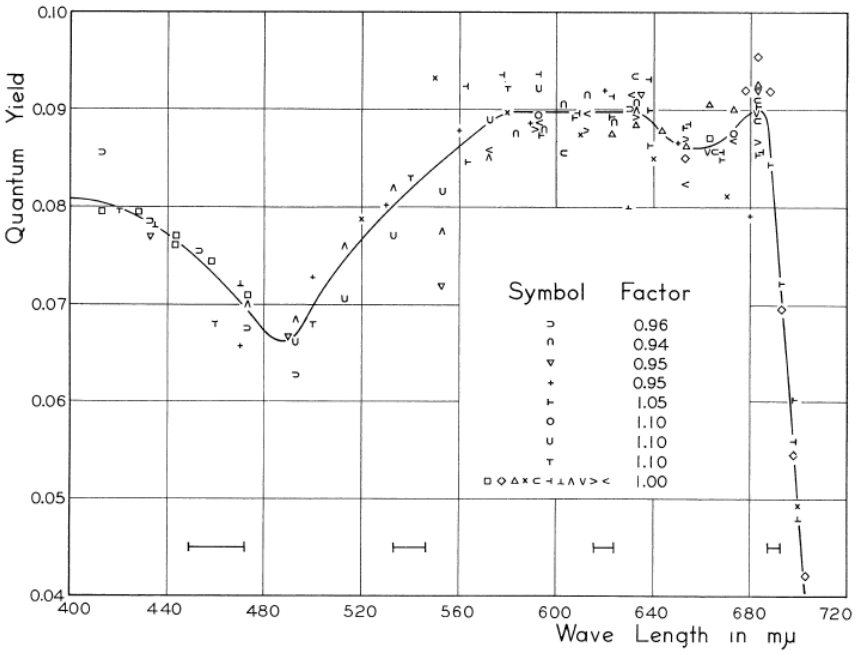

Figure IV.5: "The quantum yield of photosynthesis as a function of wave length for *Chlorella*. The points obtained on each of nineteen separate runs are indicated by a distinct symbol. For eight of these runs arbitrary adjustments have been made by multiplying all the values obtained in each run by a factor close to unity. The factors used are given in the figure. The band half widths that were commonly used in the various parts of the spectrum are indicated by horizontal lines of corresponding lengths." Reproduced from Emerson & Lewis (1943), p. 171.

the spectrum, the yield dropped sharply (see fig. IV.5). All attempts to measure photosynthetic yield in the regions beyond 700 mμ were unsuccessful. This was the phenomenon that later became known as the "Red Drop" of photosynthesis efficiency. The puzzling fact was that even at these higher wavelengths, that is, above 68 5mμ, chlorophyll absorption was still rather high, and no pigments were known to compete with chlorophyll in this region. Emerson and Lewis were completely at a loss as to how to explain this finding. They speculated "that the light quanta of this spectral region [might] no longer provide sufficient energy for the photochemical primary process";[36] yet, it seems that even Emerson and Lewis themselves were not completely happy with this line of argument. They repeatedly emphasised the preliminary nature of the measurements and refrained from making a more comprehensive explanatory hypothesis. It was only in the second half of the 1950s that scientists would finally be able to explain the findings.

4 Wartime

In December 1941, after the attack on the headquarters of the US Pacific Fleet at Pearl Harbor, Hawaii, the US entered the Second World War, which was already in its third year in Europe. This profoundly changed most scientists' research agendas, including those of the American photosynthesis researchers, so that quantum yield studies and most other projects were put aside until after 1945. The researchers reacted to the new circumstances in a variety of ways. Emerson, for example, had no desire to become involved in any directly war-related projects. Furthermore, he was thoroughly disgusted by how, from one day to the next, American citizens of Japanese origin were treated in the US. Camps were erected in which Japanese-Americans whom the authorities considered a threat to national security, were interned and deprived of any way of meaningfully spending their time and working power. Emerson used his local influence to launch a project to help alleviate this situation, at least in California. This is how Emerson described his efforts in an autobiographical note written in 1949:

> Early in the war, I became interested in rubber research, because of the importance of rubber to the United States, and also because I felt that our exploitation of Southeast Asia, where rubber and similarly important products were produced, may have played a large part in stimulating Japan to attack us. [...] I spent the war fostering a program of rubber research in the concentration camps to which the Japanese-Americans were banished. Our aim was to develop the desert shrub, guayule, as a source of rubber which could be produced under American living standards, without resort to the exploitation of native labor in Southeast Asia.[37]

To Emerson's great satisfaction, the project turned out to be a major success – not only scientifically, but also in terms of giving back content and purpose to

[36] Emerson & Lewis (1943), p. 174.

[37] Quoted in Rabinowitch (1961), p. 115. The note was written on the occasion of the 25th anniversary reunion of Emerson's graduating class at Harvard. On the guayule project, see Finlay (2009), Chapter 5, as well as the Oral History Interview with James Bonner, which can be be accessed at
http://oralhistories.library.caltech.edu/15/00/OH_Bonner_J.pdf (pp. 26–27 of the PDF file).

the lives of a number of deportees. Emerson's closest collaborator in this project was M. Shimpe Nishimura, who skilfully combined the expertise of professional gardening with his previous studies of physics at the Caltech (both of which had been brutally interrupted upon his internment). Nishimura would later become Emerson's assistant at the University of Illinois at Urbana–Champaign.

Not unlike Emerson, Charles Stacy French registered himself as a "conscientious objector", although he could only maintain this status by finding acceptable alternative occupations. As French wrote in his autobiography: "Draft dodging led me at various times into teaching elementary physics, researching chlorophyll-containing paint for camouflage purposes, and a long project on mold selection for penicillin production."[38] Other American photosynthesis researchers, however, were not as reluctant as Emerson and French to contribute to the war effort. Rieke's involvement in war research at the Radiation Laboratory of the MIT has already been mentioned, and his former superior, Franck, starting in 1942, took part in the research effort of the "Metallurgical Laboratory", set up at the University of Chicago to explore the possibilities of constructing an atomic bomb.[39] Arnold's career took yet another turn. In 1941, Arnold was made Assistant Professor of Biophysics at Stanford University. However, while he was entertaining joyful visions of "spending winters on the campus of Stanford, and summers at the Hopkins Marine Station", Arnold received a letter from Princeton University, asking him "to take part in an investigation of anti-aircraft fire", supported by the Office of Scientific Research and Development (OSRD).[40] Arnold showed this letter to the President of Stanford, who advised him to volunteer for the Princeton position. Thus, for the next four years, Arnold worked in fields unrelated to photosynthesis; and it was only in 1949, under Gaffron's prompting, that he sat down to write a paper on the results of his earlier quantum yield studies (cf. above, p. 171).

The situation was, of course, completely different in Europe. Franck's courageous resignation and his emigration to the US were outlined in Chapter III. His former assistant Eugene Rabinowitch, who was also Jewish, took the same route – from Germany via Copenhagen and London – to America, although he found it harder to obtain a new position (not least because he wasn't a Nobel Laureate). Otto Warburg's fortunes during the war were quite remarkable: he not only survived the Nazi period, he even remained, until 1945, in his original position, as the Director of the Kaiser Wilhelm Institute of Cell Physiology (founded in 1931), despite the fact that Warburg was considered "half Jewish" by the Nazis, even though Warburg's mother was of Protestant origin (so that, according to traditional Jewish law, Warburg was not Jewish at all) and his father had converted to Protestantism long before the birth of his son; in addition Warburg had always distanced himself explicitly from Judaism in general and from the Jewish part of his own family in particular. Warburg's treatment was exceptional, given the fact that so many other people of the same ancestry, regardless of their positions, were banished, deported or killed.[41] Warburg's fortune during the years of the Nazi

[38] French (1979), p. 12.

[39] See, for this period of Franck's life, e.g., Lemmerich (2007), pp. 243–251.

[40] Arnold (1991), p. 77.

[41] See, on the fate of other scientists in Warburg's discipline, e.g., Deichmann (2001*b*). The history of the Kaiser Wilhelm Society in the Nazi period has been the subject of an extended

regime has puzzled many historians. Particularly in the US, many people suspected that he collaborated to some extent with the Nazis.[42] Yet as far as is known today, no evidence for this assumption has come to the fore – if one does not include the lack of direct resistance as a form of collaboration. There is, on the other hand, ample evidence of the contempt Warburg felt for the new Government, which he largely tried to ignore. The special status of Warburg's institute, which was financed mostly by the Rockefeller Foundation, probably contributed to the fact that Warburg was, for a long time, exempted from the usual regulations within the Kaiser Wilhelm Society. Yet, in the end, Warburg most probably survived because of the continuous efforts of a number of influential friends in the fields of politics, economics and science, who repeatedly managed to get him out of difficult situations.[43]

Warburg had few illusions about his prospects as a scientist of half-Jewish ancestry in a national-socialist Germany; yet, he repeatedly emphasised that he was determined not to be dispelled "by a handful of arbitrary criminals" and that he would continue to work.[44] And so Warburg did carry on working, for a surprisingly long time, without any interruption. It was only after repeated bombing had damaged virtually all the laboratory's windows that the institute was evacuated, in the summer of 1943, to a manor in the environs of Berlin called *Schloss Seehaus* (located in the village of Liebenberg, in the district of Templin), which was completely refurbished for this purpose. One may assume that the evacuation of the institute was also done with the purpose of moving Warburg out of the direct focus of political institutions, in order to avoid further critical interest in Warburg's position.[45] Warburg himself spent most of the time in his summer residence in Nonnevitz on the Baltic Sea island of Rügen. Cut off from laboratory facilities, he mainly worked on a book, *Schwermetalle als Wirkungsgruppe von Fermenten* (Heavy Metals as the Active Group of Ferments), which summarised his earlier studies on this subject and was published in 1946.[46]

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research project headed by Reinhard Rürup and Wolfgang Schieder, the results of which were published in a series of seventeen volumes published in the years 2000 to 2007 by Wallstein Publishing, Göttingen (Germany).

[42]The plant physiologist Albert Frenkel recalled in an interview with Govindjee (on 8 Sept. 2007) that when Warburg was in the US in 1948/49, Gaffron had organised a party for him at Woods Hole, Massachusetts. It was at this party that a wife of one of the professors at Caltech bluntly asked Warburg why he had stayed in Germany "when the Nazis were doing such bad things". To which Warburg replied: "I wanted to protect my co-workers." He then added: "What could I have done?" Whereupon she replied: "You could have committed suicide!" Understandably, this shocked Warburg and many of the other guests.

[43]For more recent views on this episode, see, in addition to the biographical literature cited on p. 68, e.g., Macrakis (1993), p. 64 and p. 226 (footnote 53), and Nickelsen (2008*a*). In addition to Friedrich Glum, who was, until 1937, Secretary General of the Kaiser Wilhelm Society's administrative wing, Warburg had the support of, for example, Hermann Bücher, Philipp Bouhler, Viktor Brack, Ferdinand Sauerbruch and especially Walter Schoeller.

[44]German original: "von ein paar hergelaufenen Verbrechern"; Rüskamp (1989), p. 252; Werner (1991), p. 285.

[45]See Henning (1987), p. 85.

[46]Two short notes on photosynthesis were published in 1944, in which Warburg, together with his co-worker Wilhelm Lüttgens, published his findings that isolated (and even mechanically affected) chloroplasts were able to drive the reduction of chinone to hydrochinone, with the release of molecular oxygen. No mention was made of Robin Hill's earlier experiments – it is not

The Russian front arrived in Liebenberg in April 1945. While Warburg was still on Rügen, he spoke on the telephone almost daily to one of his co-workers, Wilhelm Lüttgens, who, together with his wife, had been taking care of Schloss Seehaus.[47] "Frequently there are air raid warnings – three times every day and every night," Lüttgens recorded on 15 April.[48] Lüttgens spoke on the telephone to Warburg for the last time on 27 April; the next day, the fighting had reached Liebenberg and on 30 April, the Red Army took over the village. During the course of the occupation, Warburg's institute in Schloss Seehaus was completely cleared out: all the instruments, chemicals, benches, furniture and glasswork were taken by the Red Army. And although later the Russian commander of the occupied zone, Marshall Shukow, personally apologised for the plundering and commanded that everything be immediately returned, the instruments and the furniture were never seen again. Consequently, the Kaiser Wilhelm Society abandoned the building in Liebenberg, which was then taken over by the local hospital. In June 1945 the original building of Warburg's institute in Dahlem was also occupied, this time by the Allied High Command in Berlin. Thereupon, Warburg dismissed all his employees. This was the end of his renowned Kaiser Wilhelm Institute (which, of course, would later be re-established as part of the newly founded Max Planck Society.[49])

From a letter that Warburg wrote to his sister Lotte on 13 January 1946, it transpires that he had already started thinking about a way out of his situation:

> I am living in my house in Gary Street again [in the Dahlem quarter of Berlin] (American Sector), and thanks to the Americans and Russians I am neither starving nor freezing. Until the end of September I stayed with Jacob [Heiss], whom I saved, with much effort, from military service and the *Volkssturm*, and who is well, in Rügen. We were then brought to Dahlem in Russian cars, with all our belongings. Four weeks ago, Marshall Shukow fetched the two horses from Rügen, and asked me, after I had eaten with him in Babelsberg, whether I had any other requests. Now, the horses are back in Düppel, and my private life has returned to normal.
>
> Less favourable is the situation concerning my scientific work. I cannot yet say what I am going to do; of course, I have received several offers. But you know, from 1933, that I am not a friend of emigration, as this means that one's quality of life, whatever happens, deteriorates considerably. For the moment, I am staying put – with an institute, if possible; if not, without an institute – and perhaps only go and work as a guest in other countries. As a guest – who will definitely, after a conceivable period of time, return to his home country – one is usually welcome. ([The saying] "fish and visitors stink after 3 days" is a bit exaggerated, if one not only eats and drinks in the host country but also does some work.)[50]

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entirely clear whether Warburg was aware of them at the time. See Warburg & Lüttgens (1944*a*) and Warburg & Lüttgens (1944*b*). See Nickelsen (2008*a*) for a transcript of Warburg's personal diary notes in the first months of 1945.

[47] A diary kept by Wilhelm Lüttgens documents in rich detail the situation in Liebenberg from April to September 1945, and has been published in Werner (1991), pp. 326–334; doc. 124.

[48] Werner (1991), p. 326.

[49] Werner (1991), p. 338.

[50] Otto Warburg to Lotte, 13 January 1946. The originally German document is quoted in Werner (1991), pp. 355–356, doc. 128.

These then were the circumstances in which Warburg found himself at the beginning of 1946. Notwithstanding the insecurity of his position, Warburg, aware of his status in science, was nevertheless optimistic. It is admirable that, even during these hard years, he never gave up his scientific pursuits. Warburg had no satisfactory infrastructure at his disposal and, given the state of the country, there was hardly any hope that this situation was to change in the foreseeable future. Once the war was over, however, Warburg immediately began catching up with the international scientific literature. One of the first things that he published, in 1945, was a short note on the quantum requirement of photosynthesis,[51] which he wrote in response to the papers by Emerson and Lewis and to a review of the subject written by Franck and Gaffron in 1941, in which they had announced that the issue had been settled in favour of a minimal quantum requirement of twelve.[52] It is hardly surprising that Warburg strongly contested this perspective, and responded by vigorously reconfirming his earlier findings.

5 The Photosynthesis Project at Urbana

Soon after the end of the Second World War, Robert Emerson was approached by the University of Illinois to set up a research laboratory dedicated to photosynthesis studies on the Urbana campus. Emerson had been looking for an opportunity to leave Caltech for some time already, yet he only accepted the attractive offer from Illinois on the condition that the university also hire a physicist or physical chemist with an interest in photosynthesis, so that the project could be properly guided in both respects – plant physiology and physical chemistry.[53] His request was granted and, in 1946 Emerson was appointed Urbana Research Professor of Botany as well as a Co-Director of the newly founded "Photosynthesis Project".

Right from the start, Emerson had envisaged Eugene Rabinowitch, who was then at the University of Chicago studying uranium chemistry as part of the Manhattan Project's Metallurgical Laboratory, as a potential second director.[54] The first volume of Rabinowitch's seminal monograph on photosynthesis had just been published, which made him a particularly eligible candidate for the position.[55] Emerson himself had not studied much physics and chemistry and greatly respected those who were competent in these fields. Yet, he was deeply sceptical about the use of theoretical speculation without empirical evidence. Emerson was fully aware of the limited reliability of even the best biological data in quantitative terms, and it was extremely hard to persuade him that, within margins of error, even imperfect measurements could be used to construct more comprehensive models. Personally, Emerson would rule out data as "n.g." (no good) whenever he believed that they had not been obtained with the greatest attention to consistency and precision in procedure. Hence, although Emerson thought that the Photosynthesis

[51] See Warburg (1945) for the paper.

[52] Franck & Gaffron (1941), p. 200: "We know now that the high quantum efficiency mentioned is only apparent, and that the true efficiency is only a third of it, namely, 12 quanta per CO_2 molecule reduced. The foundations on which the hypotheses concerning the amazing efficiency and the four-step mechanism rested have disappeared."

[53] Govindjee (2004*b*), p. 181.

[54] On Rabinowitch, see, e.g., Rabinowitch (2005), Brody (1995) and Bannister (1972).

[55] Rabinowitch (1945); the second volume was published in two parts in 1951 and 1956.

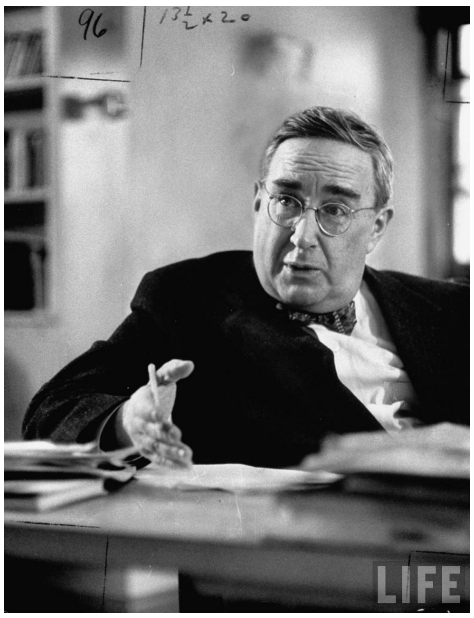

Figure IV.6: Eugene Rabinowitch (1901–73) in c. 1958.

Project also required a more theoretically oriented person, it had to be one with sufficient sensitivity towards the specific problems of biology. Rabinowitch seemed to meet these criteria.[56] On 23 October 1946, Emerson wrote to the Dean to request that Rabinowitch be appointed Co-Director of the Photosynthesis Project. He explained his choice by describing the intended scope of the laboratory's work, which, he argued, needed Rabinowitch's skills to complement his own interests and knowledge:

> If he [Rabinowitch] is appointed, it will be our plan to make a joint attack on the problem of energy absorption and conversion in the green plant. My share of the program will be the study of photosynthesis as it takes place in the intact cells of lower plants. Mr. Rabinowitch will work on artificial systems, built either from components extracted from plant parts or from non-living material, which give promise of simulating the unique energy-storing aspects of the natural process of photosynthesis.[57]

Emerson's request was granted, and thus began what Warburg would later mockingly describe (according to Rabinowitch) as the "Emerson–Rabinowitch photosynthetic unit". The arrangement was to prove highly satisfactory – the combination of their talents, characters and approaches did indeed prove fruitful, and the Photosynthesis Project at Urbana was to develop into one of the most active research centres on the subject.

[56] See Rabinowitch (1961).

[57] Emerson to Carmichael on 23 October 1946, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Botany Department. University of Illinois Archives.

6 Attempts to find a Solution

6.1 Warburg Comes to the US

On 28 November 1947, Emerson wrote his first letter to Warburg since losing touch with him after 1939. Therein, Emerson reported how he had heard, through the Urbana-based chemist Roger Adams, that Warburg had responded to the challenge of the Warburg–Negelein quantum yield values posed by Emerson and Lewis by publishing a paper. Emerson wrote: "After months, I finally obtained this manuscript from our military administrations. We translated it into English and it will be published in the *American Journal of Botany*."[58] The translation was published as Warburg (1948) and entitled "Assimilatory quotient and photochemical yield". Therein Warburg described the papers from the Madison group as being methodically flawed and the critique by Emerson and Lewis as being insubstantial. At the same time, Warburg fully confirmed the quantum requirement values of 1923, based on new measurements that he had taken using the two-vessel method.

With hindsight one can already find in this first response some of the leitmotifs of the ensuing controversy: for example, Warburg's arrogant contempt of Emerson's findings. After all, Warburg was the master who had developed the methods, which Emerson had only learned from him much later as Warburg's student. Warburg wrote that he "would be astonished if Emerson had found the truth with our methods while we ourselves had fallen into error in our application of the same method".[59] Warburg continued to explain why he considered Emerson's approach to be flawed:

> The disadvantage of this method [of Emerson's] is that the bicarbonate solutions are unphysiological from the standpoint of their chemical composition, osmotic pressure, and pH. The hydrogen ion concentration of the least alkaline bicarbonate solution is 10.0–9.4 and thus differs from the physiological hydrogen ion concentration for *Chlorella* by more than four orders of magnitude. [...] It would never have occurred to us to measure the most delicate of all biochemical processes, the conversion of light energy into chemical energy, in a medium in which the very survival of the cells seems remarkable. Not so Emerson. He reasons, correctly, that by determining the yield in bicarbonate solution all his concern about the assimilatory quotient is eliminated. He makes the unjustifiable assumption, however, that the effect of the unphysiological medium can be neglected for the sake of this methodological simplification.[60]

Warburg wrote that, in his own experiments, he had found that the assimilatory quotient γ was -0.93, which was sufficiently close to -1, even for intervals of only five minutes. Thus, Warburg maintained that "through this result Emerson's main objection to our yield determination has been refuted: the physically and physiologically improbable allegation that the photochemical reduction of carbon dioxide is introduced by an outburst of carbon dioxide".[61] Likewise, he dismissed

[58] Archive of the BBAW, NL Warburg 262; Emerson to Warburg on 28 November 1947. The "manuscript" that Emerson refers to is the German paper Warburg (1945).

[59] Warburg (1948), p. 194.

[60] Warburg (1948), p. 194.

[61] Warburg (1948), p. 195.

Emerson's second objection – that the pressure changes in the first minutes of illumination were drastically different from the remaining period: "With our new method, the method of manometric γ determination, we found nothing of the sort. With our old method, which Emerson used, we sometimes, particularly at rather high light intensities, observed small 'Emerson' effects, which quite likely originated from nothing other than bubble formation."[62] In these cases, Warburg reported, he had rejected the readings taken at the beginning of a light or dark period and only used the readings taken towards the end of the respective period. As in 1923, he still found "a quantum requirement of 4 to 5 per molecule of evolved oxygen".[63] In these passages, the second leitmotif of the controversy emerged: Warburg's habit of not answering objections head on, but rather of presenting new data that he had obtained by altering his methods (so that the critic then needed to demonstrate, first of all, whether the earlier objections also held true for the new set-up).

However, at this early stage of the controversy, Emerson chose a different strategy and made the following suggestion:

> It is now being discussed in America how to explain the inconsistency in determining the yield of assimilation. It seems to us that it would be best if we could observe the same phenomena in a laboratory together and calculate the yield in the same manner. If Germany had not been so badly damaged and if you still had your laboratory, I would suggest that I come to visit you in Berlin. But as far as I have heard, at the moment it is impossible for you to undertake any kind of scientific work. Hence, I suggest that you visit us here and carry out some comparative experiments in our laboratory. We are still far from being as well equipped as you were in Dahlem, but nevertheless our laboratory is sufficiently equipped for carrying out quantum yield measurements. You may want to bring [Fritz] Kubowitz with you and your strain of algae, a Hefner lamp and whatever other instruments need to be compared.[64]

The university's administrative department had already agreed to fund the visit, and Emerson suggested that this should also be used to cover Warburg's and his laboratory assistant's travelling expenses as well as a salary for the two of them for six months (Warburg's assistant was assumed to be his long-standing collaborator Fritz Kubowitz.). Emerson also announced that he would now apply for immigration permits from the US State Department, so that they would be ready in time.

Warburg answered on 19 December. He thanked Emerson for the invitation and said that he would come with Wilhelm Lüttgens as his assistant.[65] Warburg also wanted to bring his valet and secretary Jacob Heiss with him; Warburg would pay Heiss out of his own salary, and if necessary would also cover Heiss's travelling expenses. However, Warburg still needed a personal invitation for Heiss, otherwise

[62] Warburg (1948), p. 195.

[63] Warburg (1948), p. 195.

[64] Archive of the BBAW, NL Warburg 262. Emerson to Warburg on 28 November 1947.

[65] Emerson, of course, did not know that in 1944 Kubowitz had denounced Warburg to the Nazi authorities; Warburg had been saved thanks to some influential friends, and would never speak to Kubowitz again. Lüttgens was the only one among his long-standing collaborators whom Warburg still fully trusted. See, for background information, e.g., Nickelsen (2008*a*).

it would be impossible for the latter to travel; at the time, German citizens were still not free to travel abroad. And to enter the US, they also had to prove that they were politically unstained.

During the course of the following months, Warburg repeatedly changed his choice of assistant, his means of transport, payment and other details – all to Emerson's exasperation, since any one of these changes meant that he had to resume negotiations with both the university and immigration officials. In the end, Warburg brought only Heiss with him, and the two of them arrived by plane. Warburg was probably never aware of all the trouble Emerson had gone to in order to organise his visit. Emerson succinctly described his feelings in a letter to Gaffron on 29 May 1948:

> Dear Hans: When I saw a letter from you in yesterday's mail, I hoped it would say you planned to come down and visit us over Memorial Day week-end. The weather is perfect today, and I feel it is high time you saw our laboratory. Maybe you will come while Warburg is here. It really looks as if he would come, there is mail here for him, from Paris.
>
> [Carl] Cori [the physiologist] is right, his [Warburg's] visit is sure to lead to a lot of grief. In fact, just trying to arrange for the visit has kept me busy for a large part of the winter. After all our efforts to provide Warburg with an assistant of his own choosing, it turns out the man (Gustav Ernst Lau) cannot come because he lives in the Russian zone. Seems to me Warburg might have thought through of this difficulty a few months ago, instead of now, when he is about ready to leave. Last report I had was that he and Heiss might leave by June 1st. I hear they have 400 kilos of baggage and a poodle, on all of which they expect the Univ. of Illinois to pay transportation. It will turn out that the reason Warburg wants to leave Germany is because the American administration has been unable to get any more of that good German dog-food, made of pure beef-steak, the only thing the poodle will eat. There will be Hell to pay when he finds that in America they feed horse-meat to dogs! And imagine the problem of finding housing for Warburg, Heiss, and a poodle!
>
> Yes, I believe Cori is right, but I hope it will be worth the trouble, to get this matter settled. Bob.[66]

No mention was ever made of the poodle again, so it can be assumed that it stayed behind in Germany. Warburg, though, entered the US, together with Heiss and an enormous amount of luggage, on 26 June 1948. He left a disastrous first impression on Emerson and others, as one learns from a description recorded in an interview with the German chemist Karl Friedrich Bonhoeffer:

> Otto Warburg was traveling with [a] diener [servant] and five or six large crates of personal belongings. He was going to the University of Illinois, presumably on a permanent appointment. He was met, several hours after the arrival of the plane, by Professor Emerson. [...] Fellow travelers were either elderly German women clothed in their best black dresses and going to homes of sons or grandchildren in the United States, or young German brides of American GIs. Since the plane was over twelve hours late, practically none

[66] This letter is in private hands; thanks to the intercession of Govindjee, Peter Homann kindly made it available to the author.

> of these inexperienced travelers were met at the airport and each had their own problem: No knowledge of English, no American money, no ticket for air travel to Spokane, etc. W.[arburg] seemed completely selfish, being much more concerned with the probably undeclared gold nuggets in his baggage than with the problems of his fellow travelers. He was visibly annoyed by the fact that there were no airline officials before 7:00 A.M. to handle his possessions shipped as cargo.[67]

As Emerson was soon to experience, Warburg was never to make up for the bad impression he had made on his arrival.

6.2 The Time Spent at Urbana

When Warburg arrived at Urbana, he immediately turned the laboratory upside down. As Rabinowitch wrote in his obituary of Emerson, Warburg was accustomed to working in a laboratory that completely and utterly fulfilled his wishes; and, since Emerson's laboratory was not large enough for Warburg to have been given full command of a section, he and Emerson had to tolerate each other in the laboratory's communal areas. This was bound not to work smoothly.

However, Emerson still had high hopes that the visit would pay off. He truly believed that Warburg had come to carry out experiments with him and discuss the discrepancy between their results; yet nothing of the sort happened. Warburg proceeded to work in his own usual way and was not at all interested in Emerson or his work. The only positive reaction to Emerson's challenge that Warburg showed at Urbana was the fact that he had refined his new two-vessel technique for measuring quantum yields. However, instead of discussing these or other details of the technique with Emerson, Warburg spent most of his time constructing an actinometer: a device to measure radiation intensity by way of monitoring a chemical reaction (in this case, the uptake of oxygen produced during the chlorophyllide reaction by thiourea). This was surprising, given the fact that Warburg had up to then used bolometers, which were far more accurate.[68] In his paper of 1948, Warburg wrote that he had decided to abandon the experimental procedure of 1923, "because of the danger of frothing" (which he thought was the reason for the alleged carbon dioxide burst), and "because it is unnecessarily cumbersome".[69] Instead, Warburg suggested that light intensities should be measured by means of a chemical actinometer, which he considered "so simple that it can be used as a laboratory experiment in a physiology course".[70] Although this was certainly true, one still wonders why Warburg resorted to trading precision of measurement for ease of handling – there were certainly other ways of responding to Emerson's critique that Warburg's use of the bolometer needed to be improved (if this was indeed the motivation behind the changes Warburg made to his experimental procedure).

[67] Quoted in Werner (1991), p. 382 (doc. 147); shelf mark of the original document: Archives of the Rockefeller Foundation, r.g.1.1., s.717, b.2, f.11. The story that Warburg had tried to sneak out some valuable items in order to sell them for dollars was also circulated by Hans Krebs.

[68] Warburg had already mentioned this alternative way of determining quantum yields as a valid possibility in his German paper for the "Fiat review", Warburg (1947), pp. 209–210, and repeated this suggestion in Warburg (1948), pp. 208–209.

[69] Warburg (1948), p. 208.

[70] Warburg (1948), p. 209.

A dramatic turn of events finally occurred towards the end of the year. It is reasonable to assume that Emerson had gradually lost his patience with Warburg, and so had decided to try another way of getting Warburg to discuss quantum yields with him. In a letter to French, who wanted to meet Warburg at Urbana between 17 and 18 December, Emerson wrote:

> It so happens that we are having a number of guests that night, for a seminar discussion on quantum yields in photosynthesis, to be led by [the photochemist] Farrington Daniels at 9 o'clock Saturday morning, the 18th. There will also be a luncheon at noon Saturday, for as many of the photosynthesis people as can stay for it. I hope you will come to both the seminar and luncheon. We expect to have Rieke, Kamen, Commoner, Daniels, Stauffer, and probably some others. It is very fortunate that the date of your visit coincides with this meeting. Please let me know on what train you arrive, I'll probably be able to meet you.[71]

As can be taken from an earlier letter that Emerson had written to his colleague Martin Kamen on 26 November, it had been agreed that Farrington Daniels, from Madison, would bring down some pieces of his apparatus to Urbana for Warburg to take a look at and make comments. Emerson wrote: "Also, Warburg has proposed a procedure for measuring quantum yields upon which both of us can agree, and I am reasonably certain the results will leave him holding the bag."[72] Emerson was clearly keen to invite everybody who might be interested (and be valuable for the discussion), and the meeting, which was chaired by Daniels, unexpectedly developed into a large conference, with an audience of about a hundred, and with several speakers, besides Warburg, scheduled to give a presentation. According to Albert Frenkel, who had been an assistant to Emerson at Caltech, where the two of them became good friends, the meeting was civilised, and without the nasty quality that the controversy would later acquire; yet, nothing came out of it.[73]

The last chance to come to some sort of agreement came the day after Christmas, about four weeks before Warburg intended to leave Urbana. Warburg had finally agreed to carry out some experiments alongside Emerson and his co-workers, and have the results judged by impartial observers. The two observers were the biochemist Dean Burk, who was then at the National Cancer Institute (NCI) in Bethesda (Maryland), and a colleague of his, John Z. Hearon, who had recently completed his doctoral degree in biochemistry at the University of Minnesota. It was probably the botanist Oswald Tippo, at the time Head of the University of Illinois's Botany Department on the Urbana campus, who invited these two men to carry out this function. (Note that the gathering was documented by the local press: see fig. IV.7, p. 195.) However, still no agreement was reached during this twelve-day period of common experimentation (26 December to 6 January). Warburg

[71] Emerson to French on 3 Dec. 1948, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: French, C. Stacy. University of Illinois Archives.

[72] Quoted in Kamen (1985), p. 304.

[73] Frenkel and Govindjee (personal communication [telephone interview], 8 September 2007), and generously made available to me. See also Frenkel (1993) for an autobiographical account of Warburg and how the quantum controversy came to the Marine Biological Laboratory at Woods Hole (Massachusetts) in 1949.

Figure IV.7: A photographic record of the twelve days of conducting experiments together. From left to right: Victor Shocken, Shimpe Nishimura (face obscured), Dean Burk, Oswald Tippo (at the rear), Otto Warburg and Robert Emerson. The photograph appeared in the local *News Gazette* on 9 January 1949. (The original is preserved in the University of Illinois Archives.)

gave his side of the story in a letter he wrote to the plant physiologist Frederick C. Steward of the University of Rochester (New York State), on 2 January 1949:

> A few days ago I got cells to measure the yield in this way [i.e. by means of Warburg's recently developed actinometer] and I found, in the presence of two impartial observers, 4 quanta per molecule oxygen. Proceeding in exact [sic] the same way the next day two assistants of Dr. Emerson found for a different culture about 20 quanta per molecule of oxygen. So I came to the conclusion that the *Chlorella* cultures of Dr. Emerson are not suitable or at least unequal and I declared here that I could continue the experiments only if I had the control over the cultures of the *Chlorella*. This is a long story.[74]

Warburg went on to explain that the question could only be settled if he found another place to stay for the rest of his time in the US and inquired whether Steward would be able and willing to host him. In the end, Warburg joined Burk at the NCI in Bethesda; but before Warburg left Urbana, an official protocol, set up on 7 January 1949, documented the outcome of Warburg's stay in Urbana:

> *On the Quantum Requirement of Photosynthesis*
>
> [...] A simple manometric procedure, involving the photo-oxidation of thiourea sensitized by ethylchlorophyllide, has been developed and employed [to measure the light energy]. A new modification of the two-vessel method for determining oxygen and carbon dioxide exchange independently and simultaneously has been developed and employed [for photosynthesis measurements].
>
> With this method the photochemical evolution of carbon dioxide in certain cultures of green alga – *Chlorella pyrenoidosa* – has been repeatedly observed. In instances the ratio of carbon dioxide produced in light to oxygen produced in light has been as high as plus 4 instead of minus 1. From this it follows that the photochemical evolution of carbon dioxide could invalidate measurements of the efficiency of photosynthesis if it is not taken into account. [This paragraph was confirmed by Warburg's signature.]
>
> Our recent measurements by the new methods have given quantum requirements for oxygen production which vary from 4-6 as a minimum to virtually infinity (no yield), apparently depending upon cell conditions. [This paragraph was confirmed by Emerson's signature.][75]

Thus, in the end both parties acknowledged that under some conditions the data purported by the opponent were in fact obtained; yet no agreement was reached about how significant these data were and how they should be interpreted. Burk suggested that the text be used as the basis of a jointly authored note of the opponents and the impartial observers and that it be submitted to *Nature* with the intention of informing the scientific public about the outcome of Warburg's visit to Urbana. However, on 10 January, Emerson cabled Burk to withhold publication (or omit Emerson's name) until further changes to the text had been agreed upon.[76]

In a letter to Burk on 21 January 1949, Emerson explained his objections in more detail. First of all, he wrote, the note, as it was, left readers with the

[74] Letter generously provided by David Walker, who first reproduced this letter in *Energy, Plants and Man*, with kind permission of Geoffrey Hind.

[75] Werner 1991, S. 385, Dokument Nr. 149. (= Archive of the BBAW, NL Warburg 265)

[76] Archive of the BBAW, NL Warburg 174. Emerson to Burk, 10 Jan. 1949.

impression that it covered the entire period of Warburg's stay at Urbana: "It could be concluded that over the entire six-month period of Warburg's visit, we were never able to obtain consistent values for the quantum requirement. This implies a more serious criticism of conditions in my laboratory than is justified by the facts." Emerson, therefore, insisted that they include the fact that "the photosynthesis measurements referred to were all made during the 12 days of your [Burk's] visit". Emerson mentioned to Burk that he had tried to amend the text by making minor insertions, yet ended up completely rewriting large portions of it.

Emerson had already shown the rewritten text to Warburg, who had flatly rejected it; nevertheless, Emerson felt that Burk should also see what he himself believed was a fair statement of the facts. However, Emerson also added: "The more I think about the problem, the less I feel that any useful purpose would be served by publication at the present time. The only cogent reason for a published statement is the one given by Dean Ridenour [Louis N. Ridenour, then Dean of the Graduate College at Urbana] that it would reduce the circulation of unfounded rumors. In spite of the importance of this, I think it is outweighed by the fact that the data constitute a rather insufficient basis for the establishment of conclusions so important as those we are trying to reach. Any conclusions we draw now are subject to modification by the next few months of experimental work. This being so, I believe publication now is worse than no publication at all."[77] The following two paragraphs are those rewritten sections of the original text:

> New measurements of the quantum requirement of photosynthesis have been initiated, using the modified technique to avoid errors from possible photochemical evolution of carbon dioxide. Unfortunately, only two weeks were available for these experiments. It was during this period that Drs. Dean Burk and John Hearon were members of the group. The cultures available at this time showed widely divergent results, not only from one experiment to another, but also within most of the individual series of observations. [commentary in the margin to this last sentence: "This is absolutely untrue. Warburg"] In certain experiments several successive 10-minute light exposures gave quantum requirements of 4 to 6, both per molecule of oxygen produced and per molecule of carbon dioxide consumed. But these exposures were proceeded or followed by others in which much larger numbers were obtained, running in some instances to infinity (no photosynthesis at all). [commentary in the margin "Not true. Warburg"]
>
> These experiments raise again the possibility that the maximum efficiency of photosynthesis may be greater than the value (represented by about 10 absorbed quanta per molecule of evolved oxygen) that has become widely accepted among American investigators of the problem. However, an unambiguous and convincing determination of the maximum quantum yield of photosynthesis will require establishment of conditions leading to consistent and reproducible results. At present we are unable to offer a satisfactory explanation for the wide variability of the results reported here. The presence of appreciable bacterial contamination in all the *Chlorella* cultures used for these experiments raises the question to what extent processes other than

[77] Archive of the BBAW, NL Warburg 174. Emerson to Burk, 21 Jan. 1949.

> photosynthesis may have contributed to the pressure changes attributed to illumination.[78]

Burk did also not like the specification that the note was the result of two weeks – or rather: twelve days – of working together (he would have preferred to write that they all had worked together "for several weeks", since, Burk hastened to add, he and Hearon had spent at least two weeks each on the problem before they arrived); nor did he agree with the last two sentences, which "did not seem to me to be particularly true", as Burk stated. He closed his letter to Emerson with: "I gather [...] that you are not keen on publishing jointly at present, and would prefer the various parties concerned to go their own way separately, presumably after further work."[79]

Thus, even after Warburg had spent six months at Urbana, no resolution of the controversy between Warburg and Emerson seemed to be in sight: Warburg's four to five quanta per oxygen molecule stood against Emerson's value of ten to twelve quanta (which was in agreement with the measurements that Rieke, Arnold and Magee et al. had taken). Whereas Emerson later spoke of being thoroughly depressed at the outcome, Warburg announced his victory to everybody who would listen. "It was as [if] somebody put it [sic] here a drama watched by all America and the happy end was the victory of truth," Warburg wrote to Tippo, after having left Urbana.[80] Warburg also used every possible opportunity to belittle Emerson and his work. For example, on 21 January 1949 Warburg wrote to French, after the latter had inquired whether Warburg would not like to stay a little longer in the US:

> Certainly I have not told [said] that it is impossible to work scientifically in the US. But I have told [said] that it is impossible in Emerson's laboratory. It seems to me that many scientists in this country are aware of this; but unfortunately nobody warned me. It is no crime to make mistakes in science. But it is another thing to fight established truth for years and years strewing sand into the mills of science.[81]

In February, Warburg moved on to the NCI in Bethesda, where Burk had succeeded in securing him a six-month position (supported by the Public Health Service).

Warburg never forgave Emerson for insisting on his point. Moreoever, Warburg increasingly cast himself as the victim of a conspiracy of some American researchers – the "Midwest Gang", as Warburg would later call them. Besides Emerson and his colleagues and co-workers at Urbana, such as Rabinowitch, this "gang" also included Gaffron, Franck and Daniels. Andrew A. Benson, co-discoverer of the carbon reduction cycle in photosynthesis, recalled the following episode from an encounter with Warburg in 1952: "On a beautiful afternoon I drove him [Warburg]

[78] Archives of the Max Planck Society (MPS); III. Abt., Rep. 1, Nr. 187. Enclosed in a letter from Burk to Emerson on 25 January 1949.

[79] Archives of the MPS; III. Abt., Rep. 1, Nr. 187. Burk to Emerson on 25 January 1949.

[80] Warburg, Otto to Tippo, Oswald, 17 Feb. 1949, Personal file of O. Tippo, (former) Botany Department, University of Illinois at Urbana–Champaign, now: Department of Plant Biology. I am grateful to Clint Fuller and Govindjee for pointing out this letter to me.

[81] Archives of the MPS; III. Abt., Rep. 1, Nr. 198. Warburg to French, 21 Jan. 1949.

and Herman Kalckar to 'Hamlet's castle' at Helsingør [Denmark]. Warburg peered through an iron grate into the darkness below, 'Ach, it's a perfect place for that Midwest Gang'."[82] And although Warburg was known for his sharp tongue, keen sense of humour and even self-mockery (which he unfortunately lost in his later years), he might not have been entirely joking when he made this quip.

6.3 Woods Hole and the 1949 Papers

Bethesda and Woods Hole

After having spent four months at the NCI in Bethesda, Warburg, accompanied by Burk, moved in June 1949 to the Marine Biological Laboratory (MBL) in Woods Hole (Massachusetts), where he would spend the last month of his stay in the US. For this purpose, the whole experimental set-up for measuring photosynthetic quantum yields, including the culture vessels, Warburg apparatus, etc., was transferred for the summer from Bethesda to Woods Hole. There, another confrontation with Emerson arose during the annual meeting of the Society for General Physiology, at which Emerson and Warburg met again to discuss quantum yields. This time the opponents became more outspoken and emotional; for example, the plant physiologist Michel Burlyn, at the time attending one of the Woods Hole summer courses as a student, recalled how surprised he was by the "heated exchange between Emerson and Warburg following a presentation by Emerson, in which he disputed Warburg's claim of high efficiency".[83] Again Emerson was sorely disappointed with the outcome of the meeting. Gaffron, who was in the audience, reported the course of events to the chemist and photosynthesis expert Martin Kamen:

> On June 22 we had another round between Warburg and Emerson. The new measurements of the Warburg group were presented by Dean Burk who said, or was it Warburg himself, that never have quantum yields been measured so accurately and definitively as at Bethesda (Burk's laboratory). [...] Not only have Warburg's results been confirmed as expected but it was seriously contested that quantum numbers of 3 have theoretical significance. Emerson, of course, showed numerous experiments evidently proving that no change in procedure will bring the quantum yield below the conventional 0.1. Most among the lay audience were inclined to believe Warburg who stated that the matter was settled and that Emerson's data were wrong.[84]

Emerson himself mentioned the meeting one month later in a letter to Arnold, written on 21 July 1949 – apparently still in a gloomy state of mind:

> Dear Bill: I was sorry you didn't come to Woods Hole, but glad to pick up some news about you from [Stanley] Holt. I hope he gave you a good report of the meetings. I wish I knew what your opinion is now concerning the quantum yield of photosynthesis. Burk regards the matter as settled in Warburg's favor. I am unable to put my finger on any error in the Burk–Warburg experiments which would appear to account for the discrepancy between their results and mine, but as Franck says, there are a number of

[82] Benson (2002*b*); Quoted also in Govindjee (2004*b*), p. 184.
[83] Michel Burlyn to Govindjee (personal communication [email], 27 November 2007).
[84] Quoted in Kamen (1985), p. 304. Letter by Gaffron to Kamen dated 25 June 1949.

> things about their experiments which are "very fishy". I felt it wasn't much use to discuss things with Burk, because to me he seemed inclined to conceal important points in a rather deceitful way. I dislike having a controversy with such people. Warburg doesn't speak to me at all any more.[85]

Shortly after this last contest, Warburg returned to Berlin, where he succeeded in having his institute re-established as one of the newly founded Max Planck Institutes.

The *BBA* Paper

On reading the sources, it becomes clear that, apart from the factual discrepancies, the controversy had, at a very early stage, acquired an element of mutual lack of trust, personal defamation and suspected dishonesty. Warburg seemed to have taken Emerson's critique as a personal slight, while Emerson in turn felt exceedingly offended by Warburg's dismissive attitude towards him. This situation was not alleviated by a paper submitted in June 1949 to the *Biochimica and Biophysica Acta*, co-authored by Warburg, Burk, Victor Schocken and Sterling Hendricks.[86] A first reading confirms the impression that Warburg and Burk were intent on changing their strategy by taking the controversy (which was basically centred on one item of data) to another level, namely, using scientific esteem and prestige as a weapon; for example, they richly decorated the paper with photographs that had no obvious relevance to the content but rather showed the authors at work or posing with a number of other eminent scientists, mainly Nobel Prize laureates. Warburg et al. admitted in their paper that the established value of no more than four quanta of red light to produce one molecule of oxygen, has "sometimes been doubted by theoreticians, and it is a fact that certain investigators have raised methodological objections" (p. 335; note that in the main body of the paper no mention was made of Franck, Gaffron or Emerson, who are only cited in the Appendix. The accusations in the argument of the paper were vague and not directed at any person in particular.) Notwithstanding these critical voices, the authors strongly confirmed the accuracy of a minimum quantum requirement of four, which they had obtained again by using far more simplified experimental methods. The bolometer had been replaced by the actinometer; they placed much stress on the "kind and rate of shaking" (p. 339), since the authors believed that only a specific procedure would safeguard the cells from being mechanically affected, which would lead to lower quantum yields. Warburg again raised the charges that he had made previously against Emerson's findings – that the cells had been poorly treated and that frothing had occurred because the solution had been inappropriately shaken.

The new technique advocated by Warburg et al. was a two-vessel method, in which a slightly acidic, phosphate-containing buffer solution was used. The use of a two-vessel technique was a response to a criticism raised by Emerson and Lewis, who had asked that the changes in carbon dioxide and oxygen should be measured simultaneously, while the acidic medium remained unchanged. Warburg et al. had also devised a procedure that, from their point of view, made all calculated

[85] Emerson to Arnold, 21 July 1949, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Arnold, William. University of Illinois Archives.

[86] Warburg, Burk, Schocken & Hendricks (1950).

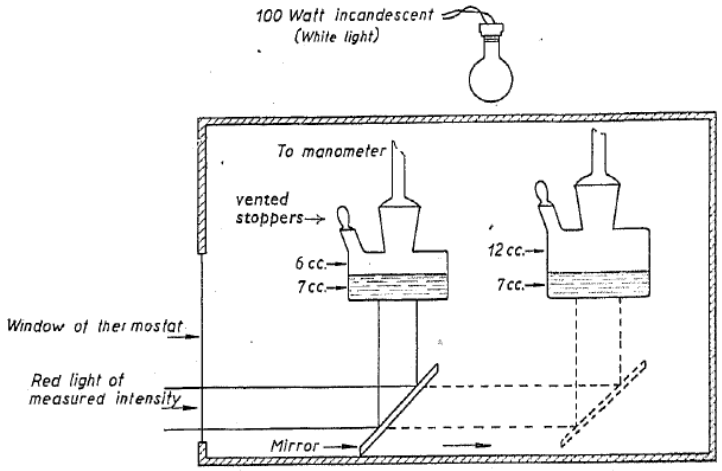

Figure IV.8: Warburg's experimental set-up in 1950: a beam of red light (630-660 nm) of about 3-4 cm^2 in area entered the side of the thermostat and was reflected by a mirror onto the bottom of one of two vessels, alternately in the one or the other, by shifting either the mirror or the manometers. It was assumed that the red light entering the vessel was completely absorbed (this was ensured by using thick cell suspensions). Additionally, the vessels were continuously illuminated with white light from above, which kept the process of photosynthesis at a high enough level to compensate for respiratory gas exchanges. Reproduced from Warburg, Burk, Schocken and Hendricks (1950), p. 338.

corrections for respiration effects unnecessary. In their set-up (see also fig. IV.8, p. 201), they used white light of undetermined intensity that illuminated the vessels from above, which ensured that the rate of photosynthesis was always far more intensive than the rate of respiration; an additional red beam of measured intensity caused an increase in the rate of photosynthesis, which was then manometrically recorded. Warburg et al. assumed that the light of the red beam was completely absorbed by the cells. Thus, Warburg et al. underlined that they no longer had to work near the compensation point, at which photosynthesis and respiration almost equalled each other, and which rendered all manometric measurements rather complicated and delicate. In addition, the new set-up was praised for the fact that, in contrast to earlier measuring techniques (which typically could only yield reliable data for a period of less than one hour), the yield experiments could now be continued for up to ten hours, and still yield efficiencies of three to five quanta per molecule of oxygen. Finally, the authors made a point of stating that the quotient of CO_2/O_2 (γ), which they claimed to have monitored simultaneously, was between -0.8 and -1.3; this was presumably done to counter Emerson's criticism of variable γ values, although they failed to mention Emerson, his paper and the specific critique.

It was only in the Appendix that Warburg et al. dealt explicitly with the objections raised by Emerson and Lewis. The authors explained that Emerson and Lewis had used a carbonate-bicarbonate buffer solution only in order to escape the difficulties that were encountered when using a phosphate buffer solution (notably, the carbon dioxide burst!), and that Emerson and Lewis believed that the actual photosynthetic quantum yield was the same in both solutions. Warburg et al. complained, however, that no data had been provided to substantiate this statement. Thus:

> We can confirm Emerson's finding that in the carbonate-bicarbonate mixtures the quantum requirement is 10 to 12, but we cannot confirm that the same quantum efficiency is obtained in the acid culture medium. [...] Maximum yields should therefore not be determined in the carbonate mixture, as has been done frequently during the last 10 years.[87]

The *Science* Paper

The BBA paper was soon followed by a second one, framed as a "re-discovery" of the high efficiency of photosynthesis. The list of authors was slightly altered, with Burk named as the first author, and it was published in *Science* in September 1949.[88] Therein, the authors explicitly acknowledged the use of the facilities of the MBL at Woods Hole, and particular mention was made of Professor Eleazar S. Guzman-Barron and Director Charles Packard. The *Science* paper's content did not substantially diverge from the *BBA* paper outlined above, although in the conclusion the authors stated:

> It follows from the data obtained that in the spectral region 630 to 660 mμ no more than 4 quanta are required to produce one molecule of oxygen gas. A requirement of 3 quanta is open to serious consideration, although thus far the average value in our experiments has been nearer to 4 than 3.[89]

The interesting fact again is that in a carbonate buffer solution the quantum values measured by the Warburg group were 10.5, 9.8 and 11.3 – thus, nicely matching the measurements done at Urbana and elsewhere. The low values of 3.6 or 3.9 were only obtained in an acidic phosphate-containing buffer solution. Unsurprisingly, this did not go unnoticed, and Burk et al. commented on these measurements: "It may be gathered from this example that the efficiency in the unnatural carbonate buffer is only a fraction of the efficiency in culture medium" (p. 228).

This paper was widely read and received considerable attention. Burk, Warburg's man in the US, wrote to his new master and mentor on 2 November 1949:

> The *Science* article has been a grand success, and it is too bad that you have not been here to enjoy all the attention that has been paid to it. We have had an enormous number of requests for reprints from every corner of the country and every kind of laboratory, including, oddly enough, many hospital

[87] Warburg et al. (1950), p. 346.

[88] The Science paper was published before the paper in the BBA, although it had been submitted some months later.

[89] Burk, Hendricks, Korzenovsky, Schocken & Warburg (1949), p. 229.

> laboratories. There have been many popular write-ups, with more to come. News Week [sic] ran a column on it under the heading 'Battle of the Plants'. Industrial and News Edition of the American Chemical Society is planning a 3000 word lead article. Scientific Monthly has asked me to write an article on the subject, of general nature. Time Magazine is considering running an episode. Even the Washington Evening Star of last Thursday (eight weeks after appearance of the article) ran a column on it, and described the work as 'one of the major scientific events of the year'.[90]

Public attention would not die down for a long time, as it was being constantly fanned by Burk's series of public speeches, which included his talk at the annual meeting of the American Association for the Advancement of Science, which took place at the end of December 1949. In his speech Burk framed Warburg's research in economic terms, and confronted his audience with a calculation of how much more energy could be retrieved through sunlight if the photosynthetic process could be exploited on an industrial scale.

The Paper in *Archives of Biochemistry*

Warburg and Burk only dealt explicitly with Emerson and Lewis's critique in a third paper, which was published in the *Archives of Biochemistry* in January 1950.[91] In this paper Warburg and Burk maintained that ...

> ... the proposed 'CO_2 outburst', at variance with the experience of the previous century and a half, was never actually demonstrated in published experiments involving quantum yield measurements. In the only completely detailed efficiency experiment in which the light-dark time course was repeated [cited Emerson & Lewis 1939, p. 4], a calculation, not performed by the authors, shows that the pressure changes for the second 5-minute periods of illumination were actually more positive (less negative) than for the first 5-minute periods [...]. This result, offered as a typical experimental example, was a direct contradiction of an outburst.[92]

Getting this paper published in the *Archives* had not been an easy task. On 18 December 1949, Burk informed Warburg that "after some difficulty" the paper had been accepted, although the editors had recommended that the referees' advice for revision be followed.[93] Burk wrote a letter of explanation to the editor (which had the intended success) and changed nothing.[94] However, Burk was not sure how to

[90] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 2 Nov. 1949.

[91] Warburg & Burk (1950*b*).

[92] Warburg & Burk (1950*b*), p. 413.

[93] The anonymous referee report is preserved in the Burk–Warburg correspondence folder, which is held by the Archive of the BBAW; see the sheet dated November 9, 1949, NL Warburg 174. Publication was recommended, despite the fact that the paper was very one-sided. Summarising experimental results rather than writing them out in detail was requested. Of particular interest is the following statement: "The emphasis that these experiments have been conducted by students in the physiology class appears unfair to those who have had experience with students and who know how easily younger people can be influenced by a strong personality. This should not be taken as a criticism of the experiments as such, but it appears to me absolutely unfair to quote any inexperienced person in support of work which even an experienced investigator has great difficulty in forming a clear picture of the essential points which may modify his results."

[94] Burk's reply is likewise preserved; see Archive of the BBAW, NL Warburg 174.

address the problem of Emerson's value; until then he would have firmly endorsed Warburg's notion that inadequate shaking had irreversibly harmed Emerson's cultures, while now he believed that he had some evidence to suggest that the shaking might, after all, not be so important. Burk's solution was characteristic of his general attitude towards the controversy:

> I think we should be careful not to indicate that Emerson's 'Emerson Effect' was due only to inadequate shaking on his part, even though that might indeed be one way to produce such an apparent effect. The less said specifically about Mr. Emerson, the better I believe.[95]

On the whole Burk was enthusiastic about this piece of work: "The more I read the article, the more I like it, everything is so beautifully clear and well organized, and it is a classic in its way."[96] It is worth noting that this paper was written after the 1949 volume *Photosynthesis in Plants* had appeared, edited by Franck together with the plant physiologist Walter J. Loomis, in which, for example, Arnold's paper was published. Warburg and Burk directly addressed the challenge raised in the 1949 publication: "The results reported in this volume seemed to be conclusive and final: with three different and independent methods – manometric, polarographic, and calorimetric – minimum quantum requirements of 10-12/molecule of O_2 produced were obtained." (p. 414) However, Warburg and Burk were determined to contest these results based on their experimental findings at high light intensities, the many advantages of which they discussed at length (pp. 419–421). Nine experiments were described *in extenso*; again, in their only attempt to use a carbonate buffer solution, Warburg and Burk faithfully reproduced Emerson's quantum yield values.[97] The authors conceded this phenomenon, yet again drew attention to the fact that it only occurred in a medium that they found unacceptable because of its alkaline conditions.[98] In a large table at the end of the paper, Warburg and Burk finally detailed the values of $\gamma = CO_2$ absorbed/O_2 produced in their experiments, ranging from -0.8 to -1.33 with an average of -1.06. These deviations, they pointed out, were totally at variance with what Emerson and Lewis had claimed:

> On average a little more CO_2 was absorbed in the light than O_2 was produced. Thus, one of the two main loopholes that have been used to evade the high efficiency in photosynthesis is now closed. [...] The fact must thus be envisaged that in a perfect nature photosynthesis is perfect too.[99]

It is hardly surprising that Warburg (together with Burk) and Emerson no longer communicated directly with one another. However, after the publication of the *Science* paper, Emerson turned to the plant physiologist Sterling Hendricks (one of the authors of this third paper), whom he had met earlier in his career, and asked him how he could possibly have joined forces with Warburg and Burk. The

[95] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 18 Dec. 1949.
[96] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 18 Dec. 1949.
[97] Warburg & Burk (1950*b*), "Experiment No. 2", pp. 432–433.
[98] "In no experiment in carbonate buffer at pH 9 have we observed lower quantum values than 8, the average being about 10"; Warburg & Burk (1950*b*), p. 441.
[99] Warburg & Burk (1950*b*), p. 413.

latter wrote to Warburg, on 2 November 1949, stating that he had spent Thursday evening with Hendricks, helping him to answer Emerson's letter:

> ... [Emerson] spent many crocodile tears in the letter over the fact that Hendricks had ever had the misfortune to get himself tied up with such "dishonest" work as ours, in which many unsupported claims were made, and in which many formerly claimed virtues of procedure were now relinquished without explanation: settling of cells, low intensity, slow gas stream etc. Then Emerson went on to say that our cell concentration was now too great to be properly handled by the kinds of vessels we used. (You remember in his 1949 book article he had complained otherwise, that you had earlier used too small a number of cells?). Well, now there are too many, according to him, to even get good respiration readings. [...] Hendricks did not try to answer many of the detailed points, but told Emerson he thought they were all minor, and that he ought, if interested, to try and find something wrong in major principle, if he could! So Emerson is, as we fully expected, up to his old tricks. But he won't get far.[100]

It is worth taking a look at this from the other side of the dispute. Emerson wrote the following letter in response to a report of Burk's presentation at the American Association for the Advancement of Science (AAAS) Annual Meeting at the end of December 1949 by the plant physiologist Frederick C. Steward of the University of Rochester (New York State):

> Dear Mr Steward,
>
> I appreciated receiving your account of Burk's performance in New York. One of our graduate students was there, too, and gave us his impressions, but the field is so new to him that he couldn't give us as full an account as you do. We are amused that Burk had no time for discussion of results with scientific colleagues, but had plenty of time to spill a big story for newspaper reporters. [...]
>
> Yes, Burk gives one this impression that he is making an intentional effort to confuse issues, rather than to clarify them. I'm inclined to agree that an ethical problem is involved, as well as a question of scientific fact. I'll appreciate advice on how to deal with the ethical issue, but I'm inclined to let it go until we have settled the facts.
>
> With best wishes,
> Sincerely
> Robert Emerson.[101]

6.4 Franck's Attempt to Find a Compromise

While Emerson struggled to find the errors in Warburg's experimental procedure and calculation method, Franck chose a different strategy. He was ready to grant Warburg that his data and methods were as sound as Emerson's, yet Franck suggested that these data did not reflect the maximum yield of actual photosynthesis. On 14 March 1949, when Warburg was still in Bethesda, Franck sent Warburg a manuscript to look at, with the following remark:

[100] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 2 Nov. 1949.

[101] Emerson to Professor F.C. Steward January 28th, 1950; Botany Department; University of Rochester Rochester 3, New York. Letter kindly provided by David Walker.

> I would be so glad if you could subscribe to the view that the differences between the findings in the quantum yield rather indicate a difference in the observed photochemical processes than to some measurement error on the part of the observer.[102]

Franck wanted to publish this hypothesis and, was, therefore, interested in hearing what Warburg had to say. Two weeks later, Warburg answered rather briefly that he had studied the paper but, of course, could not agree; furthermore, Warburg did not want it mentioned in the paper that he had read the manuscript, since people might then assume that he supported the paper's argument. However, Warburg concluded the letter on a warm note: "Finally, I have to say how very glad I was to see you again. Our last meeting was in Berlin, at the Physical Society, seventeen years ago, when you made your unforgettable speech in memory of my father."[103]

Franck's paper was published shortly thereafter, in the *Archives of Biochemistry*.[104] Therein he briefly reviewed the disagreement, describing how Emerson and Lewis explained Warburg's earlier measurements: namely, that in a acidic phosphate-containing medium there occurred an outburst of carbon dioxide, which resulted in values of very low quantum yields – possibly even lower than four. This explanation, Franck underlined, had become widely accepted in the US. However, Franck also mentioned that, in the meantime, Warburg had rejected Emerson's criticism and reconfirmed his values under rather different experimental conditions. Franck continued:

> The present writer, who was privileged to have oral discussions with Warburg and with Emerson, finds it hard to accept the point of view that only Warburg's method under special conditions will permit the algae to reduce CO_2 with a quantum yield of $1/4$ when all other observations systematically give $\sim 1/10$ as the highest value. On the other hand, it is not certain that the occurrence of the Emerson effect [i.e. the CO_2 outburst] is the main cause of the difference between Emerson's and Warburg's new results, because this effect, while undoubtedly present in both observations, seems to be smaller in Warburg's new experiments than in Emerson's.[105]

From Franck's point of view, the difference by a factor of two between the values of the two parties seemed too high to be entirely due to the confounding factor identified by Emerson and Lewis; furthermore, Franck saw some evidence pointing to the fact that Warburg's findings might not only be quantitatively different from those of other groups but also qualitatively. Franck's suggestion, then, was intended to reconcile the results of Emerson's and Warburg's measurements, under the assumption that respiration might be interfering with the different processes of photosynthesis – in particular under conditions where the latter is not much higher than the former, that is, around the compensation point, for example, at very low light intensities:

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[102] Archives of the MPS, III. Abt., Rep. 1, Nr. 195. Franck to Warburg, 11 March 1949.
[103] Archives of the MPS, III. Abt., Rep. 1, Nr. 195. Warburg to Franck, 28. March 1949.
[104] Franck (1949).
[105] Franck (1949), p. 298.

> We introduce the assumption that Warburg's high quantum yield may be connected with the reduction of respiratory intermediates rather than with the reduction of CO_2. That is possible because Warburg's measurements are carried out under conditions where the photosynthetic rates are smaller than or, at best, comparable to, the respiration rates.[106]

Although other researchers had suspected that respiration interfered with photosynthesis, no systematic attempt, Franck believed, had been made to explore the consequences of this observation; he was convinced that his approach was "not only able to reconcile Warburg's quantum measurements with those of others, but also agrees with results of an entirely different nature".[107] In particular, Franck suggested that, instead of using CO_2, the reducing agents of photosynthesis might be utilised, under certain conditions, to reduce half-oxidized respiratory intermediates:

> Thus, every half-oxidized molecule reduced photochemically will prevent the consumption of one-half molecule of oxygen by respiration. The evolution of CO_2 will be diminished by a whole molecule. In other words, the process would add one oxygen molecule and take away one CO_2 molecule from the balance sheet of respiration for every 4 quanta absorbed by the photosynthetic apparatus.[108]

Franck then pointed to recent findings that indicated a strong affinity of chlorophyll to the acid respiratory intermediates, if only they were able to enter the chloroplast. Franck suspected that Warburg's experimental conditions might result in an abnormal permeability of the chloroplast membrane, which would allow respiratory intermediates to enter these organelles in great quantities. The culture's age might be one of those factors, prolonged anaerobicity another factor, and so forth.

However, after developing this argument in rather sophisticated terms, Franck conceded, in a "note added in proof", that in light of the experimental conditions used by Warburg, Burk and others at Woods Hole, this theory of his had become obsolete – because in these experiments, the yield remained high even under conditions where photosynthesis exceeded respiration several times, thanks to the combination of white background illumination with a red beam of measured intensity. In addition to that, at very low carbon dioxide concentration levels in the vessel, illumination with the red beam "did not measurably change the oxygen consumption of normal respiration".[109] Far from being frustrated, Franck critically observed:

> However the present author is still convinced that the above discussion contains a good deal of material useful for the reconciliation of the differences in the results of quantum yields and of the chemical nature of intermediates of photosynthesis. He believes that there are two different photosynthetic processes, one with the quantum yield of $1/4$, the other with 8 quanta and that

[106] Franck (1949), p. 299.
[107] Franck (1949), p. 299.
[108] Franck (1949), p. 300.
[109] Franck (1949), p. 312.

> the former is exceptional, taking place only when the chloroplast membranes become permeable, thus permitting mutual interactions between photosynthesis and respiration. High acidity, which discharges acids, is obviously one of the conditions necessary for the occurrence of that process.
>
> If, as the new experiments indicate, the replacement of CO_2 reduction by that of half-oxidized respiration products is not responsible for the higher quantum yield process observed by Warburg, it might be that a part of the energy of respiration can be used for photosynthetic processes. [...] [I]t might be possible that the energy stored in the phosphate bonds produced by respiration might be transferred to phosphate bonds of the CO_2 complex and of intermediate products of photosynthesis. In that way, the energy of 12 K-cal. would be available in the molecules to be reduced before each photochemical reaction and, with that additional energy photosynthesis may proceed with 4 quanta. However, this photosynthesis could, even if all other conditions are favorable to it, only proceed to a maximum rate of 1.5 times that of respiration. Any photosynthesis in algae beyond 1.5 times respiration would need 8 quanta.[110]

The idea that back reactions might be involved, which, to some extent, linked respiration and photosynthesis, or, alternatively, that the energy gained from respiratory processes was used for photosynthetic carbon dioxide reduction, would be around for the next few decades and would become very influential. In fact, even though Warburg himself would have shuddered at this thought, one could claim that this idea became a major source of inspiration for Warburg and Burk's later photosynthesis model, which became known as the "one-quantum mechanism" of photosynthesis (see section 7.1).

6.5 Metaphorical Blows are Exchanged

Although Emerson was in rather low spirits by the end of 1949, he was far from being defeated. In his letter to Steward of January 1950, partly quoted above (p. 205), Emerson also outlined his future research plans:

> As I may have suggested in my last letter to you, we are now pretty sure that the major cause of error in the Burk–Warburg work is their assumption [...] that there was no "physical lag" in their manometric system. On the basis of this unsupported assertion (which we find to be strictly contrary to fact), they based most of their photosynthesis measurements on 10-minute exposures to light, alternated with 10-minute intervals of either darkness or unmeasured light. This does not lead to correct values of photosynthesis in either phosphate or carbonate buffer solutions. However, there are additional factors in their work which are still obscure, and we shall not be satisfied until we can give a quantitative explanation of all the major inconsistencies. We do make progress toward this objective, but it's disappointingly slow.[111]

Emerson pursued these line of research with his assistant Nishimura and with Charles Whittingham, a postdoctoral student from the University of Cambridge (United Kingdom), who was spending a year at Urbana.[112] Emerson presented

[110] Franck (1949), p. 313.

[111] Emerson to Professor F.C. Steward, January 28th, 1950; Botany Department; University of Rochester, Rochester 3, New York. Letter kindly provided by David Walker.

[112] See Nishimura, Whittingham & Emerson (1951) for the pertinent publication.

the results at a symposium on "Carbon Dioxide Fixation and Photosynthesis" in July 1950, organised by the Society for Experimental Biology and hosted by the Biochemistry Department of the University of Sheffield (UK). The conference was attended by all the major players in the field of photosynthesis, including, among others, Franck, Emerson, Gaffron, French, Robin Hill, Bessel Kok, Melvin Calvin, Daniel Arnon and Burk. This group of excellent speakers was complemented by some renowned figures from the discipline of biochemistry, such as Hans Krebs and Harland G. Wood, who were working on the phenomena of carbon dioxide fixation in heterotrophs. Warburg was also expected to attend, although he failed to show up at the last minute. Emerson gave a detailed report of his journey to Europe in a letter of 3 November 1950 to the physicist Louis N. Ridenour, who was, at the time, Dean of the Graduate College at Urbana; Burk also wrote a relatively long letter to Warburg about the Sheffield symposium. Thus, the event is well documented by both.

The largest part of a full day of the symposium was devoted to the discussion of the minimum quantum requirement of photosynthesis, with centre stage being given to Emerson's and Burk's presentations. According to his letter to Ridenour, Emerson was very satisfied with his experiences there. He felt that never before had he spoken to an audience so "clearly willing to give its attention to the experimental details that have come to play such an important part in this controversy". Close attention was given to both sides before the floor was opened for questions:

> [George E.] Briggs did a careful job of steering the discussion, and of giving opportunity for expression of all viewpoints. Burk was asked to give his opinion regarding the criticisms of his work which were implied by the tests of his methods reported from our laboratory. At first, he tried to avoid comment, but questioners were insistent, and he finally said there seemed to him to be nothing in our work which he would not be able to explain in a few days' time. I think it is fair to say that the consensus of opinion was that he had failed to prove his case.[113]

It is interesting to compare Emerson's letter with the letter that Burk wrote to Warburg immediately after the Sheffield symposium. Needless to say, his account diverged quite widely from Emerson's:

> Veni, vidi, but not quite vici. The Philistines were present in great numbers, including all our old "friends" who surpassed themselves in their attempts to muddy the waters without any new experiments. Emerson talked as in Chicago 1947, Urbana 1948, Woods Hole 1949 style, followed by a pecking at our large *Archives* article and finally by just one slide of new data, with the 2-vessel method in which he claimed that he could now get 4 quanta for certain time periods but 8 quanta if he took 30' periods and included the first 5-10 minutes. In other words, almost but not quite a confirmation yet, but a marked change, nevertheless, as [Daniel] Arnon rose to point out.[114]

[113] Emerson to Ridenour, 3 Nov. 1950, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Graduate College Correspondence, University of Illinois Archives. George E. Briggs was Professor of Botany at the University of Cambridge.

[114] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 10 July 1950.

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Burk's admission that he had not quite "won" strongly supports Emerson's impression that Burk had failed to convince the audience. It is also interesting to observe that Burk saw the situation as a matter of victory versus defeat, while Emerson, by contrast, mentioned a lack of proof. It also seems that Burk had failed to grasp Emerson's point, since the latter had in fact attempt to show under which circumstances one would get the (artefactually) high quantum yield. In the remainder of the letter, Burk emphasised that everybody in Sheffield was greatly disappointed that Warburg had not shown up, and that people kept asking Burk whether Warburg might, perhaps, make it after all. Burk had also shown photographs of the Dahlem laboratory during his talk, which, he thought, greatly impressed everybody – most of the participants had assumed that the institute was still in ruins. Burk proudly boasted: "I think it made the old-time Berliners homesick and the British and American keenly interested."[115]

The contributions that were so greatly discussed in Sheffield were published in a conference volume one year later. In Emerson's contribution, co-authored by Nishimura and Whittingham, the authors very clearly explained why the two-vessel method, used by Warburg and his co-workers for their quantum yield measurements, was extremely sensitive to very significant systematic errors. Even tiny aberrations, which in the one-vessel method would be trifling, were likely to have enormous consequences on the final result. As Nishimura et al. argued in great detail, slight errors in the individual manometer readings – errors of no more than 0.3 mm, which were bound to occur all the time – could dramatically change the calculated values of γ and γ^{-1}, which, in turn, severely altered the resulting quantum yield. They identified the most dramatic source of error as being the fact that, in contrast to long established practice, Warburg and Burk had, in their measurements, failed to take into account a time interval for a physical lag in the response of the manometer to the change from light to darkness and from dark to light. While Warburg and Burk had claimed that "in their experiments mixing was so efficient that there was no physical lag in the response of the manometer to the successive light and dark periods", from the point of view of Nishimura et al., there was clear evidence that a physical lag occurred and that it had substantial effects on the readings.[116] Consequently, Nishimura et al. concluded that the measurements provided by Warburg et al. failed to demonstrate the efficiencies which they reported. The authors were not impressed either by the values for γ provided by Warburg and his co-workers in the various articles (which were always near -1): if the value was calculated separately for light and dark periods, as Nishimura et al. demonstrated, large deviations from -1 were revealed.

In view of the technical difficulties inherent in manometric techniques, it is surprising that the authors still thought the method was indispensable.[117] Nishimura et al. argued that the two-vessel measurements should be developed with a differential manometer and with the simultaneous illumination of both vessels (at the time, the two vessels to be compared had to be measured one after another, which,

[115] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 10 July 1950.
[116] Nishimura et al. (1951), p. 194.
[117] See Nishimura et al. (1951), p. 178.

of course, increased the likelihood of an experimental error). Only this would allow for readings to be taken with the required degree of precision. In their conclusion, the authors made the following comment on Warburg's strategy of experimentation and argument:

> We find in Warburg & Burk's data no evidence to persuade us that their specifications for obtaining highest efficiencies of photosynthesis are significant, regardless of what future experiments may prove. We note that some of their specifications are directly contradictory to the requirements emphasized by Warburg up to 1947. They mention the use of chloride in the culture solution, the importance of growing the cells in unsterilized medium prepared with well water, the avoidance of sedimentation, the temperature of 20°C being favourable for highest efficiency, etc. We find in the papers of Warburg & Negelein (1922, 1923) and of Warburg (1947) quite other specifications for obtaining highest efficiency. No chloride was added to the culture medium, and it was categorically stated that sedimentation during culture growth did not affect photosynthetic efficiency adversely. A temperature of 10°C was specified as essential for obtaining maximum efficiency. Up to 1947, it was stated that shaking the cells in a manometer vessel results in progressive damage, and ultimately in zero photosynthesis. In 1950 it is stated that cells are only damaged by shaking in darkness, and that they may be shaken indefinitely in the light without suffering injury. In no case are these specifications supported by experimental evidence. Possibly they represent only *ad hoc* assumptions.[118]

What Emerson did not include in this paper was his own nagging suspicion that, although he was reasonably sure that Warburg and Burk's methods were flawed, he was not yet fully in control of the situation. This can clearly be seen to be the case in the following letter that Emerson wrote to Gaffron on 4 April 1950, which also nicely illustrates the many practical difficulties of making quantum yield measurements, beginning with the problem of mounting the appropriate source of light:

> It still seems to us that difference in physical lag [...] is likely to be the most important source of systematic error, but we have spells of worrying that there is something else which we have not thought of yet. One can get quantum requirements of 4 without too much difficulty, but we are not yet able to get this result regularly, combined with a γ value close to unity, as Warburg and Burk claim to have done. I suspect the trick is to have just the right combination of CO_2 burst and physical lag. The errors from these 2 factors seem to work in opposite directions, when you use Burk–Warburg vessel volumes and 10'light-10'dark cycles without allowing any interval for physical lag.
>
> The light source is still causing us concern. After months of effort to get two vessels equally illuminated by fluorescent lamps, we gave it up, and tried to produce two equal beams. Individual vessels differ too much to get equality from a surface illuminated by fluorescent lights. Also, we were worried that the Burk–Warburg results might depend on having only a small spot illuminated.
>
> We made a nice beam-splitter with a ribbon filament lamp, and were able to adjust the two beams to equality within less than 1 per cent, but the

[118] Nishimura et al. (1951), p. 209.

> infrared could not be filtered out well enough to permit use of the actinometer. So we gave up the ribbon filament, and are trying to do the same thing with a little cadmium lamp. This gives barely enough energy, and is sufficiently free of infrared, but its life-time is very short, and the energy keeps dropping from hour to hour. Sometimes I'm at my wit's end to think how to get around all the difficulties.[119]

6.6 Controversial Themes Around 1950

This was the state of the debate around 1950. As is obvious from the course of events, interest in the question of the minimal quantum requirement or maximum quantum yield was still exceedingly high among photosynthesis researchers. In 1941, Franck and others had been convinced that the question had been settled in favour of quantum requirements approximating ten to twelve quanta per molecule of oxygen evolved.[120] However, in view of Warburg's reaction after 1945, the general photosynthesis collective was no longer so sure.

The controversy was surely influenced, to some extent, by factors "outside" scientific boundaries *sensu stricto*. Warburg was the Nobel Prize laureate, with a well-founded reputation as being an excellent experimenter, mastering manometry to the point of perfection; Emerson was considerably younger, far less famous, and, although he was hardly less proficient than Warburg in manometry, he had learned his technique in Warburg's laboratory, so that he always carried the stamp of a "disciple of", with the obvious connotations of inferiority. These were the circumstances upon which Warburg and Burk never failed to dwell, in order to gain maximum advantage out of them; furthermore, Burk was not adverse to using other unpleasant rhetorical tricks.[121] However, while their influence cannot be disputed, it is not so clear how far these circumstances actually drove the course of events. It is obvious that the general public, manipulated by journalists who had been thoroughly worked on by Burk (and who were more interested in crisp headlines than in going through dull experimental minutes), tended to think that Warburg was right and that the matter had been settled once and for all in his favour. And to a certain extent this also held true for the scientific public not involved in photosynthesis research. Yet, even the "inner circle" of scientists, who actually engaged with the subject matter themselves, many of whom were on excellent terms with Emerson, gave serious consideration to the new arguments brought forward by Warburg and Burk – not because of Warburg's reputation, but because the question was so important and the answer so obscure. The experiments from which the value had to be derived were so delicate that anyone could err, Warburg as well as Emerson.

[119] Emerson to Gaffron, 4 April 1950, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Gaffron, Hans, University of Illinois Archives.

[120] One reads, for example, in the paper Franck & Herzfeld (1941): "All theoretical considerations about the chemical nature of the intermediate products of photosynthesis and all thermochemical conclusions, therefore, have to be radically changed, since the number of photochemical steps and correspondingly, the energy balance is changed by a factor of 2 to 3." (p. 978).

[121] Note, e.g., that Warburg and Burk constantly only referred to Franck as the main opponent to Warburg's work. As a quantum physicist, Franck allegedly argued on theoretical grounds only, while Warburg himself let Nature speak. If Emerson was mentioned at all, he was usually belittled. E.g., in Warburg, Geleick & Briese (1952), Emerson was introduced as a "botanist" (in other words, as someone who could not possibly know much about manometry).

With hindsight, it seems extraordinary that the photosynthesis researchers repeatedly revisited the theme and over such a long period, discussing at length the merits of one experimental approach compared with another as well as the many possible interpretations of the sets of data – particularly in view of the fact that the "accurate" value (of eight to twelve light quanta per molecule of oxygen), had been around since the start of the controversy in 1937. It was, in particular, two factors that potentially confounded the results, which were extremely difficult to control and made it so hard to judge the validity of quantum yield experiments: these were the condition of the algae cultures and the influence of respiration. It was already clear then that both factors decisively influenced the outcome of manometric experiments (although it was far less clear to what extent and in which ways) and it was known that the combined effects of these factors with other parameters, such as light intensity or temperature, might yield further unforeseeable consequences. All this had been positively established by Emerson and Lewis in 1939, at the latest. Yet, being aware of the difficulties did not mean that they had been resolved.

The choice of medium was another factor that influenced the outcome, although it was much easier to control the medium than the internal, physiological state of algae cultures or the obscure interfering effects of respiration. It has been repeatedly emphasised in this chapter that the choice of an acidic phosphate-containing buffer solution (preferred by Warburg and Burk) versus an alkaline, carbonate-bicarbonate buffer solution (preferred by Emerson) was significant. Emerson and Lewis had provided ample evidence that the use of an acidic buffer solution favoured the development of a carbon dioxide burst, which tended to distort the experimental findings towards apparently lower quantum requirements. Warburg, on the other hand, argued that only the lowest possible quantum requirement was of theoretical importance. He had no trouble reproducing the values arrived at by Emerson and others using an alkaline medium; yet, Warburg held that, since the lowest numbers could only be obtained using a phosphate buffer solution, the measurements taken in carbonate-bicarbonate buffer solutions were useless. From Warburg's point of view, the higher values in an alkaline medium were caused by the fact that the algae were unable to photosynthesise properly at higher pH values.[122] The issue remained controversial, so that around 1950 Emerson's priority was to establish once and for all that the data obtained in an alkaline buffer solution were as valid as the data arrived at using an acidic medium.

Returning to the physiological state of the algae as one of the two main confounding factors mentioned earlier, one can clearly see from the correspondence between the major players how deeply they were preoccupied by this problem. Cultivating the algae had top priority, and was one of the most time-consuming activities in photosynthesis laboratories of the period; indeed, Emerson employed a person specifically for that purpose, while he closely corresponded with the most knowledgeable algae experts of the time, such as the mycologist Ernst Pringsheim. Standard strains were eagerly exchanged between photosynthesis laboratories, as

[122]Note that it has already been pointed out – in Govindjee (2001) – that, initially at least, Emerson and Warburg did not argue about the values measured in a carbonate-bicarbonate buffer solution. However, this situation changed in 1952, when Burk announced that the same low quantum yield had been measured in new carbonate mixtures (see p. 224).

well as those strains of algae with which standard experiments – such as the Emerson and Arnold flashing light experiments – had been carried out. This was the only way to try and approximate the homogeneity condition with regard to the algae factor. However absurd Warburg's claim – that he was unable to work with Emerson's cultures, which were improperly grown – might look at first sight, it was not unfounded. The differences that the type of tap water (Urbana versus Baltimore tap water) had on the results suggests that growing the algae at a very special location, such as on a north-facing Berlin-Dahlem windowsill, as Warburg claimed, might very well exert some decisive influence on the algae's photosynthetic performance.[123] Indeed, the subject was so complicated that by 1950 many were inclined to believe that almost any factor connected with algae culturing might influence the eventual quantum yield.

The problem of respiration multiplied these uncertainties still further. Basic manometry was unable to differentiate qualitatively between the different kinds of gases that were produced or consumed. And although more sophisticated approaches that tried to amend this deficiency eventually became available (such as the two-vessel method), in the case of photosynthesis the matter was further complicated by the fact that respiration likewise involved changes in the volumes of oxygen and carbon dioxide; and it was also beyond question that respiration continued in the light, so that the rate of photosynthesis somehow had to be distinguished from that of respiration. The usual assumption was that the rate of respiration was the same in the light and in darkness, so that the gas exchange values in the light could be corrected rather easily by subtracting the values for respiration measured in the dark. In addition, Emerson and Lewis had tried to limit the risk of having the respiration rate changed by using short intervals of light and darkness (which, however, had to be delicately balanced: if the intervals were too short, induction periods with their transient gas exchange phenomena tended to introduce measuring errors of another kind). From today's vantage point, it is clear that the fundamental assumption was inaccurate: light *does* influence the rate of respiration. However, scientists at the time were not so sure – all they knew was that the matter was uncertain and obscure. They acknowledged the problem and were clearly troubled by it, but no solution was at hand and, hence, they carried on their work without solving the issue.[124] Furthermore, there was the additional complication that respiration, as well as many other physiological processes in

[123]Note, however, that it was claimed in Warburg, Krippahl & Schröder (1956) (p. 237) that the most efficient cultures were grown in pleasant summer temperatures, on a south-facing windowsill, which makes one have misgivings about the established causal relevance of one location over another.

[124]See, e.g., a letter by Gaffron to Emerson on 4 March, 1950: "The 2-vessel method does not allow one to find out whether the respiratory quotient is changed during the exposure to light. What you obtain is always a composite figure. I am now worrying whether it may be possible that some queer intermediate products of photosynthesis are formed which at very low light intensities are preferentially burned again, replacing part or all of the ordinary dark metabolism. We then would have a special compensation reaction for which we have no proper correction in the measurements of the dark metabolism." However, on 4 April 1950 Emerson answered: "If the respiratory quotient changes in the dark, then there is really no sense in trying to measure the quantum requirement at low light intensity. I agree with you that this is a possibility to be considered, but up to now we have all worked on the assumption that the respiration is not much changed by illumination. We should not give this up without good reason." Both letters: Robert

the cell, possibly interfered with photosynthesis in other ways than through gas exchange, such as, for example, the exchange of intermediate products; perhaps there was a systematic interaction, but even if this was not the case, the risk of artificially induced interference, which is what Franck suspected, by the choice of cell treatment or other experimental conditions, clearly existed. These were serious concerns and nobody was able to rule out that any one of the plethora of metabolical side reactions and by-products of respiration and other processes under certain conditions interfered with (and confounded) the process of photosynthesis. As was mentioned at the end of Chapter III, by the end of the 1930s scientists were well aware that photosynthesis was a complex, flexible and highly adaptive process that was influenced by a vast number of factors, most of which were still unknown.

To cut a long story short: it was extremely difficult to judge the quantum requirement calculations of photosynthesis, based on experimental data with living cells; but this did not mean that it was impossible to make sound judgements. Quantum requirements of eight to twelve had not only been measured manometrically, most prominently by Emerson and Rieke; they had also been arrived at independently by other methods, such as micro-calorimetry and polarography (the latter carried out by Frederick S. Brackett), which clearly was a fact in favour of these values. This was even conceded by Warburg himself. Yet even if this convergence of values decreased the odds that the Warburg–Negelein–Burk value was the accurate one, these other methods also faced enormous difficulties, so that the potential certainty that one could have expected from the confirmation of a value by different experimental approaches was, in this case, only moderate. And even with hindsight, one has to admit that a certain amount of scepticism was justified. After all, even when mass spectroscopy became available, as the only reliable means to double-check the course of respiration in the light and in darkness, Allan Brown, the Madison-based expert in this technique, was unable to find any influence of light on respiration.[125]

Finally, I would like to draw attention to Warburg's strategy for dealing with Emerson's critique. Rabinowitch, in his obituary of Emerson, described this as follows:

> [T]he aim of finding a complete explanation of Warburg's results proved elusive, because Warburg, rather than investigating thoroughly the conditions under which the alleged high quantum yields could be obtained, kept publishing increasingly startling new observations, whose relation to his own earlier findings was not always clear, and which made Emerson's control experiments obsolete faster than they could be performed.[126]

In his early studies, Warburg had emphasised that low light intensities had to be used in relatively short experiments, which led Franck to suggest that respiration

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Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Gaffron, Hans, University of Illinois Archives.

[125] The pertinent data were published in, e.g., Brown (1953) and Brown & Good (1955), although photosynthesis researchers knew of the data before this date, from talks and informal means of communication. On Brown's life and work, see, e.g., Black & Mayne (2006).

[126] Rabinowitch (1961), p. 123.

was interfering with the apparent process; however, by 1950 Warburg claimed, together with Burk, that the highest efficiencies were obtained at high light intensities over long periods of time. In the early studies, very thick suspensions, which needed to be allowed to settle down in the vessel, had been recommended, while later Warburg favoured the use of thin suspensions, which were rather vigorously shaken. Even the technique of supplementing a white background illumination with a red beam of measured intensity was dropped a few years later in favour of a "catalytic" amount of blue light. The concentration of carbon dioxide necessary for the highest yields was also slowly increased, up to ten per cent.

These regular changes in experimental conditions, which were already sarcastically commented on by Nishimura, Whittingham and Emerson (see quote above, p. 211), lie at the core of the controversy. Warburg failed to demonstrate the actual causal relevance of the factors implemented in his experiments; and this is why he finally lost his case. Nobody can blame Warburg for going back to his original set-up, in view of Emerson and Lewis's critique, and of changing it accordingly. But contrary to expectations, to conventions of scientific behaviour and to the requirements of methodology, Warburg never acknowledged that he had introduced changes as a consequence of appropriate criticism, well-founded comparative studies or other sound reasons. And neither did he produce sufficient evidence for the actual causal relevance of the new conditions. The changes that Warburg introduced to the set-up in later years appear completely arbitrary, were done without any explanation being soffered, and went from one extreme to the other. Emerson himself, as well as Rabinowitch, understandably interpreted this as a way of escaping methodical criticism.[127] At the very least, it was strange that the range of experimental conditions used by Warburg and his co-workers did not evoke a matching range of experimental results. The quantum requirement of 2.8, at which Warburg finally settled, was not substantially lower than the value of three that Emerson and Lewis had already found in 1938, so that one might think – as Nishimura et al. did – that the variation of parameters was, after all, not as influential as Warburg had claimed. However, proving the causal irrelevance of a factor was also impossible in this particular case – even more so when one takes into account that the papers by Warburg, Burk and their co-workers usually did not provide very precise descriptions of their set-up, even though the controversy was centred around these details.

The question remains, then, as to whether this promoted the modelling process at all. At first glance, the yield was meagre. Many financial and personal resources were consumed without the actual goal – settling the magical parameter – ever being reached. At second glance, however, these studies did result in much new knowledge, which became relevant in rather unforeseeable contexts (such as the finding of the Red Drop of photosynthetic efficiency), being amassed. Furthermore, the attempts to explain the differing results led researchers to explore aspects of photosynthesis that had so far been neglected, such as the complex relationship between photosynthesis and respiration, and the intricate details of algae cultivation. However, it was also around 1950 that people started to lose interest in

[127] On this point, see also the summary of the (inconsistent) conditions used by Warburg when demonstrating quantum yields of four and less provided by Rabinowitch (1956), pp. 1947f.

the debate, feeling that it had become stuck in a dead end. Warburg had failed to demonstrate the causally relevant factors in his set-up, and Emerson had inevitably been unable to prove them conclusively wrong. The shortcomings of the available methods – above all the technique of manometry – had clearly been brought to the fore, so that, on this basis, a solution hardly seemed attainable. Nevertheless, the proponents would continue to struggle (unsuccessfully) for some years yet.

7 A Hardening of the Fronts

7.1 The One-Quantum-Mechanism

In the autumn of 1950, following an extended stay of Burk's at Warburg's institute in Berlin, Warburg and Burk outmatched themselves by proposing the so-called "one-quantum mechanism" of photosynthesis. This mechanism was first published in two short notes written in German,[128] although an extended version was presented in the form of a paper published in *Scientific Monthly*, co-authored by Burk together with Jerome Cornfield and Martin Schwartz, in October 1951.[129] It is interesting that, in the English-speaking world, this mechanism was only published in a semi-popular version; and it was only in Warburg's review of photosynthesis in *Science*, published in 1958, that the one-quantum model made its way into a high-ranking journal for original papers.[130]

The 1951 paper started off with a derogative account of Warburg's critics, in which, again, the names of the people involved were omitted (Warburg had even failed to mention that it was Emerson who had initiated his visit to the USA), while many general and vague accusations were raised. It was also emphasised again that the criticisms directed at Warburg and Burk were based purely on theoretical considerations, and that no experimental weight had been given to the objections. Thus, most of the work done at Urbana and elsewhere was simply ignored, and the debate was distortedly featured as an argument between the open, experimental approach to science, in which nature was allowed to speak for herself (represented by Warburg), and the anthropocentric pondering of general possibilities, prejudiced by current physical theory (the approach allegedly taken by Warburg's opponents). Furthermore, Burk et al. reported that Warburg's opponents had claimed that

[128] Warburg & Burk (1950*a*) and Warburg (1951).

[129] Burk, Cornfield & Schwartz (1951). This paper was entitled: "The efficient transformation of light into chemical energy in photosynthesis: An Application of the Einstein Law of Photochemical Equivalence to Living Organisms"; therein, it was claimed that Warburg had repeatedly discussed the problem of photosynthetic quantum yields with Einstein: "Einstein asked Warburg to reconsider the matter with him should he ever succeed in finding an underlying one-quantum process such as both men felt must occur." (p. 216). However, no traces of these discussions have survived, no mention of them was made elsewhere, so that, in view of the generally exaggerated style of the paper, it is by no means certain that these discussions did indeed take place.

[130] See Warburg (1958). Note that there was considerable dismay among photosynthesis researchers following the publication of this very one-sided review of Warburg's. See, e.g., the letter by Norman Good to Robin Hill, dated 2 Jan., 1959 (Cambridge University Library, Ms. Add. 9267/J.62): "What did you think of this summer's article by Warburg on photosynthesis in Science? The general reaction in America was one of considerable irritation, in no small part irritation with the Editor of Science. The article was considered, rightly it seemed to me, as a mass of willful misrepresentation and as such not worthy of a reply. However, there are many profound regrets that scientists working in other fields should be so misinformed by a journal purporting to serve all scientists."

a minimum of ten to twenty quanta would be required to produce molecular oxygen, which was only true for the very first paper by Manning et al., as the usual alternative to Warburg's value of three to four were figures in the range of eight to ten. One undoubtedly gains the impression that no attempt was made to present an impartial account of the disagreement; instead, the authors deliberately portrayed the events as a travesty, which favoured Warburg's side of the story and made the outcome seem self-evident. Burk et al. then went on to celebrate the fact that the four-quantum requirement had been finally confirmed beyond any doubt at Bethesda. No mention was made to any of the critical responses to the four-quantum requirements; instead, they continued to discuss developments that took place in Berlin:

> Although the four-quantum requirement was now definitely re-established, the fundamental mystery still remained, as to how four low-energy quanta could cooperate to yield high-energy carbohydrate in a manner that was in harmony with Einstein's photochemical equivalence law. If a single quantum of red light furnishes some 40'000 calories per mole, where do the missing 70'000 calories (110'000 – 40'000) come from? It was clear that some unknown principle of nature must be involved that would solve this "quantum riddle of photosynthesis" in a special manner. The answer to this riddle was finally obtained late in 1950 in continued joint investigation carried out in Berlin-Dahlem.[131]

The important phenomenon was, the authors reported, an effect that up to then had escaped everybody's notice: as the intervals of light and darkness were made increasingly shorter, down to one minute each, during the dark periods following a period of high light intensity a very large amount of oxygen disappeared from the system, ten times more than during normal respiration. Burk et al. explained this phenomenon as follows:

> It thus became obvious that the actual photochemical reaction is only detectable under conditions of rapid alternation of light and dark intervals, and why it had never been observed under conditions of continuous illumination. The oxygen consumption in the back reaction (most probably not identical with ordinary respiration) prevented one from seeing the full magnitude of the forward photochemical production of oxygen. The over-all process of photosynthesis clearly consists of two different reactions which interlock cyclically and normally hide each other. One reaction is photochemical and proceeds in the light alone, and the other is a chemical oxidation reaction that goes on not only in the dark, but as further experimentation showed, in the light also.[132]

This led the authors to announce that "the quantum requirement was found under optimal conditions to be one".[133] The main idea was that the 70,000 missing calories were provided by back reactions that took place during the dark phase, in which two-thirds of the previously produced oxygen would be consumed in oxidation reactions. According to Burk et al., this also demonstrated that "water

[131] Burk et al. (1951), p. 216.
[132] Burk et al. (1951), p. 216.
[133] Burk et al. (1951), p. 216.

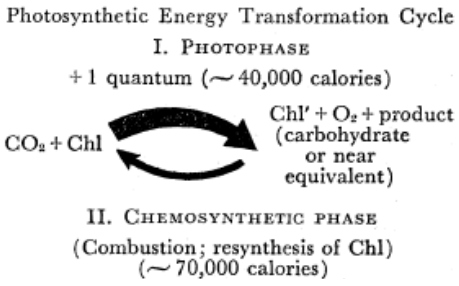

Figure IV.9: Schema of the photosynthetic cycle envisaged by Burk et al. (1951, p. 217). Chl. represents the chlorophyll complex before illumination and Chl.' the chlorophyll after alteration by illumination. Chl' is restored to Chl. by the back reaction, at the expense of the energy derived during the chemosynthetic reaction involving the consumption of O_2 and a product.

as such is not an initial reactant", but rather entered photosynthesis only during the "dark reaction", which also provided the energy required for the decomposition of the water.[134] The energy thus released would then be used in the subsequent photophase, in which the complex of chlorophyll and a carbonic acid derivative would receive another 40,000 calories in the form of one absorbed quantum of red light. In summing up, then, 110,000 calories were available to produce actual carbohydrates and molecular oxygen. An integrated circle of dark and light reactions would require three light quanta, while the photochemical process alone needed one light quantum only. The high rate of back reactions kept the efficiency at about 100 per cent. The culmination of the argument came when the authors triumphantly exclaimed: "What could be simpler than that nature, in harmony with Einstein's photochemical equivalence law, has one molecule of chlorophyll absorb one quantum of light to reduce one molecule of CO_2 and produce one molecule of O_2?"[135]

These were the findings that Burk brought back to the USA when he returned at the end of January 1951 after his stay at Warburg's laboratory in Dahlem. Upon his return, Burk immediately assured Warburg that he would "start the propaganda campaign in regard to the 1-quantum results", as quickly as possible. This included him getting in touch again with his contact (William Laurence) at the New York Times, who had promised to feature the story prominently. Burk had also scheduled many talks; the first one would be in Pittsburgh (Pennsylvania) and would be entitled, "The resolution of the quantum problem in photosynthesis". Burk, who had already sent off reprints of the German notes to all his friends and enemies, wrote: "I hope that all of this activity will strike you as appropriate to the importance of the underlying work. It seems necessary and won't take too much

[134] Burk et al. (1951), p. 217.
[135] Burk et al. (1951), p. 222.

time." And, of course, Burk was also relieved to see that all his superiors at the NCI were so delighted by the paper on the photosynthesis mechanism, "that from all signs no questions about 'cancer' will be asked, so there looks like no trouble here, on any such score".[136] (This alluded to the fact that Burk had applied for a leave of absence with the declared purpose of carrying out cancer research with Warburg, which, of course, he had not done at all.)

The reaction of the press was remarkable. Burk informed Warburg that newspaper clippings from all over the country, as well as from Germany, had reached the NCI. Furthermore, Burk had been asked to prepare a report on the energetics of photosynthesis for the National Congress's meeting on the future use of natural resources. Besides Burk, only Melvin Calvin, the Berkeley-based chemist, who was working on the photosynthetic dark reactions, and the eminent photochemist Farrington Daniels, had been asked to contribute. Burk explained that this would lead to the enormous proliferation of their findings: every congressman and senator would receive a copy, which amounted to about 10,000 people altogether.[137] It is worth taking a short glimpse into the scope and style of the newspaper reports on the one-quantum mechanism. The *Deutsche Zeitung*, for example, featured the headline: "The chemical miracle plant: Sensational discovery is considered to banish famine." The author of the article expected that the Warburg–Burk findings would fulfil two dreams of mankind: the eradication of hunger and becoming independent of coal, petrol and wood: "The industrial production of a technological-artificial plant is about to be envisaged – an innovation which would revolutionize the world's economy." The Berlin-based *Tagesspiegel* trumpeted in a similar manner on 4 March 1951: "The solar power station of nature. Berlin scientists solve the energetic mystery of the growth of plants." On 3 May, 1951, Laurence of the New York Times joined in: "Plant life study yields new data. Science team reports 'cyclic' process in use of sunlight – new substances held key." In the article Laurence reported that the discovery of the one-quantum mechanism was the "crowning achievement of his [Warburg's] life", and compared photosynthesis compared to the pilgrim step, that is, "three steps forward and two steps back". Burk's still bolder plans for the future were also quoted in this article:

> Now that the knowledge is available of the mechanism by which the green light captures its energy," Dr. Burk said, "the great problem is to find out what substance it is that first picks up the energy in one-quantum lots." Once this is known, it may be possible to devise ways of carrying out photosynthesis by chemical and mechanical means independently of plants. A sun energy factory might then produce a power that would easily replace fuels such as coal, oil, gas and wood. It might also be the basis for synthetic foods.[138]

As a result of all this public enthusiasm, it is not surprising that the NCI was, for the moment, appreciative of Burk and his achievements. And, as Burk wrote to Warburg, his superiors even defended him for not having carried out cancer

[136] All quotes: Archive of the BBAW, NL Warburg 174. Burk to Warburg, 2 Feb 1951.
[137] All quotes: Archive of the BBAW, NL Warburg 174. Burk to Warburg, 23 March 1951.
[138] New York Times, Thursday, 3 May 1951.

research: on the strength of this extremely successful sideline of Burk's, Congress should nevertheless support cancer work.[139]

Although the response of photosynthesis researchers was not quite as enthusiastic, the papers were still eagerly received. In April 1951, Burk wrote to Heiss that Franck was most interested in the large dark combustion phenomenon and had said that, "if this is definitely established something quite new and unexpected has been discovered, even if I would like to keep an open mind as to the interpretation".[140] Burk received another tentatively positive reaction from Gaffron, who, according to Burk, had conceded that "if the new observations must be interpreted as Warburg and you do, they mean of course a revolution in our general concepts. [...] I think the matter is so exciting that I would like to repeat the experiments."[141] In a similar vein, although very critical of the publication itself, Hill wrote to Emerson, on 28 April 1951:

> Whittingham and I had a talk about the Burk & Warburg note. [...] You know, unless one had done the B&W experiments oneself it seems impossible to see exactly where the experimental results are from the description. We could not see at the moment how phases can be sufficiently sharply defined in a 1 min alternation of relative or added light & dark. [...] However the Burk Warburg note was very stimulating – and yet did not seem to me to be really sharply focused on any small aspect, like the Papal Bull. And now it leaves the field quite clear for all specific studies. Warburg could do wonders with the purely biochemical parts, of course.[142]

It is obvious from his response to Hill that Emerson was far less charitable in his evaluation:

> There isn't much use in trying to discuss the Burk–Warburg 1 minute–1 minute measurements by mail. [...] I don't feel the need of further stimulation such as the new Warburg–Burk paper. I'm still confused as to the proper direction for my own further efforts. I would like to return to some of the problems raised by the Emerson-Lewis measurements at different wave lengths, particularly the sharp drop in efficiency toward the infrared, and the question whether excitation of chlorophyll with "blue" quanta can produce reactions differing in some fundamental way (higher efficiency?) than excitation with "red" quanta. To do this, I would be inclined to go back to single vessel measurements in carbonate mixture, but I feel that Warburg has put upon me a sort of curse, that I may not do this unless I can show beyond doubt that the efficiency measured in carbonate is not inferior to the efficiency in acid phosphate.[143]

One could hardly ask for a more explicit formulation of Emerson's sincere wish, by that time, to detach himself from the quantum yield controversy and return to more productive work instead. Yet the "curse" would remain in place for a number of years to come.

[139] BBAW Archives, NL Warburg 174. Burk to Warburg, 23 March 1951.

[140] Quoted in: Archive of the BBAW, NL Warburg 174. Burk to Heiss, 3 April 1951.

[141] Quoted in: Archive of the BBAW, NL Warburg 174. Burk to Heiss, 3 April 1951.

[142] Hill to Emerson, 28 April 1951, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Hill, Robin, University of Illinois Archives.

[143] Emerson to Hill, 8 May 1951, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Hill, Robin, University of Illinois Archives.

7.2 Burk's Photosynthesis Wars

In order to get an idea of the undercurrents of the controversy, in particular on Warburg and Burk's side, it seems worth taking an even closer look at how the course of events was reflected in the (large amount of) correspondence between the two. The feeling one gets is that these two actors saw themselves as the victims of an outrageous conspiracy; and that they increasingly started to believe the stories that they had made up about the qualities of their own work and the work of their main opponents. Already in his first letter to Warburg, after the latter had returned to Germany after his stay in the US, Burk assured his newly found mentor: "I am carrying on the photosynthetic war with undiminished vigor."[144] And this assertion was to be taken quite literally. In his letters to Warburg, Burk repeatedly framed the controversy on the maximum quantum yield as a series of "battles" that he had to fight, while the students, whom Warburg and Burk had met in Woods Hole and convinced of the validity of their methods and findings (among them, for example, Martin Klein, Martin Schwartz, Victor Schocken and Mitchell Korzenovsky), were referred to by Burk as their "converts" and "missionaries". In his November 1949 letter, Burk wrote to Warburg:

> And now what about our student converts? They have been kept very busy, in Chicago and Wisconsin, as you can see by the enclosed recent letters I have received by way of report. Emerson spoke in Chicago on Oct. 8, and requested that Klein and Michel be present as "Warburkian" experts, and then a little later Klein himself gave a seminar on our work, and Schwartz then gave one in Wisconsin with results as indicated in the letters, which I enclose copies of, as I am sure that you will find them of interest. How lucky it is that our Woods Hole-trained missionaries are located right in the two main camps of the enemy. They do seem to be stalwart champions to the best of their ability. It looks as if we are going to have just no trouble from Wisconsin. Emerson is evidently still busy trying to pick holes in our work but doing nothing constructive, and apparently nothing for us to worry about. Franck, although no longer on the same ship with Emerson, and having relinquished most of his former objections, is still trying to find some new ones, and Gaffron is as hopelessly confused as ever. But none of this adds up to much.[145]

Burk also liked to speak of his "propaganda work": he was kept extremely busy, driving around the country to spread the "message"; and there was considerable interest. As I explained in an earlier section, most plant physiologists knew very little about the subject; indeed, even most researchers working directly in the field of photosynthesis found the subject obscure. The experimental set-up was complicated and could only be mastered if one was extremely skilled in the technique of manometry, and the uncertainty of the outcome was large. Equally large was the interest by others in the field to see the question resolved; or, at least, have somebody explain to them what was at stake. Burk regularly informed Warburg about visitors to his laboratory, who came to see the incredible values actually being measured. French was among the first: "French was here all day Wednesday,

[144] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 2 Nov. 1949.
[145] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 2 Nov. 1949.

and his mind was at first back in the year-ago Urbana meeting, but rest assured that he was duly worked on," Burk wrote to Warburg in December 1949.[146]

It is not clear how French himself regarded this meeting. However, the slightly distorted way in which Burk perceived events (or, at the very least, reported them to Warburg) can be taken from almost any one of the letters that make up their correspondence, if one either reads between the lines or compares the account with other sources. One example should suffice. In November 1951, Burk told Warburg that he had met, at Stanford, the Dutch photosynthesis researcher Bessel Kok, and had invited the latter to come to Bethesda for the rest of the year.[147] Kok arrived on 19 November 1951, and two weeks later, Burk wrote to Warburg:

> As you may know, he [Kok] has doubted our work, with some good reason, to a greater extent than anyone else, because he has done so many experiments both before and after Sheffield, and always obtained nothing better than 8, for all practical purposes. He has been in the Spoehr-French laboratory of the Carnegie Institution of Washington at Stanford, California, since May, and they have paid his expenses to come here for a month.
>
> It took him two weeks to get here, for enroute he stopped off to see all our dear friends at Urbana, Chicago, Madison and Minnesota, at some of which places he heard that not only were all our experiments very sloppy but that you and I were very deficient in character, lying and cheating being one of our minor vices! In fact I was portrayed as being no scientist but merely a good advertising man, and the gossip picked up about you cannot be put down on paper. Kok had expected to see me literally sweat for four weeks, trying, but never succeeding, to reproduce the 4-quantum figure, so he said. To express it all in another manner, he was the shining knight of these people.
>
> All of this, both the science and the character assay, is entirely changed. In our very first experiment together, with acid media under the old 1949 Bethesda conditions, we produced a figure of $3\,\frac{1}{2}$, and in the course of the next two weeks we were able to show Kok everything, including good yields with very thin and very thick suspensions, at, above, and below the compensation point, and both in acid media and in your new pH 8.8 carbonate medium. The last results, because of their great simplicity, were naturally the most striking. [...]
>
> At the end of this, Kok said that he had seen everything he had wanted us to show him, and that he would admit to seeing the figure of 3-5 quanta such as he had never really seen in any of his own work. [...] So, we have completely changed over our biggest scientific doubter. It remains to be seen what the effects of his remarks to our other dear friends will be. Perhaps you can make a good guess.[148]

Kok's own reports, however, had a very different tone. Although he did admit to having seen the low quantum yields, he was far from being "completely changed over". Rather, he became thoroughly convinced that Warburg and Burk had misinterpreted their data. After his visit to Bethesda, Kok went on to Chicago and discussed matters with Gaffron and Franck; there he defended the view that, if one

[146] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 18 Dec. 1949.

[147] See on Bessel Kok, e.g. Myers (1987) and Renger & Govindjee (1993), a special issue of *Photosynthesis Research* dedicated to the memory of Kok.

[148] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 11 Dec. 1951.

took into account the energy of the auxiliary light (which he thought Warburg and Burk had not properly done), this led to efficiency calculations of only about 20 per cent.[149] Furthermore, in a letter to Burk sent in January 1952, Kok reported how he had discussed the data back at Stanford with "all the gods from Pacific Grove, Berkeley, etc."; it was agreed there (in line with the earlier suggestion made by Franck) that the data demonstrated "that a four and an eight quantum process are observable in one and the same suspension!". However, which of these processes actually was photosynthesis was a matter of intense debate, Kok wrote. Kok himself preferred to think of photosynthesis as the eight-quantum process, and interpret the four-quantum process as respiration suppression.[150] It is highly unlikely that Kok would have spoken to Burk, while he was at Bethesda, in completely different terms, so that one may conclude that either Burk deliberately misled Warburg, in order to take the credit for acquiring a new "convert", or that Burk tended to misunderstand what others said on the subjects of quantum yields, when they did not wholeheartedly approve of his and Warburg's work.

Note, however, that, in the letter to Warburg quoted above, Burk referred to a "new pH 8.8 carbonate medium", which had been demonstrated to Kok, in which Warburg and Burk claimed to have measured the same low quantum yields as in the usual acidic phosphate buffer solution. This effectively protected the new measurements against Emerson's (and his co-workers') unremitting criticism that in a phosphate buffer solution the data were distorted by the carbon dioxide burst. No burst had ever been observed in a carbonate buffer solution; this confounding factor was, therefore, not relevant to Warburg's new data.[151]

7.3 The Gatlinburg Conference on Photosynthesis, 1952

One obvious effect of the disagreement between Warburg–Burk and Emerson–Franck–Gaffron et al. was that it increased enormously the frequency with which formal and informal meetings between the various photosynthesis researchers took place. Burk's travels around the country to promote the Warburg–Burk cause have already been mentioned as well as the fact that he eagerly invited people to his laboratory so that they could witness the experiments with their own eyes. Kok was only one of these guests. The desire either to meet personally or to discuss the situation in extended letters also rose dramatically among the major players of the opposing party. One letter by Emerson, arbitrarily selected from the many letters comprising his correspondence, may serve as an example. On 8 May 1951, Emerson wrote to Gaffron:

> Dear Hans:
>
> Thanks very much for the time you and Franck took yesterday to talk with us over the phone and tell us the news about Burk, Brackett, etc. We hear from [Sol] Spiegelman that Burk made a convincing impression at Cleveland. It's interesting that his blatant self-advertising which offended people in England seems not to antagonize people in this country.

[149] Cf. the letter Emerson to Whittingham, 5 September 1952. Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Whittingham, Charles, University of Illinois Archives.

[150] Archive of the BBAW, NL Warburg 174. Kok to Burk, January 1952.

[151] For the pertinent publications, see Warburg et al. (1952) and Warburg (1952).

> Of course, I'm very sorry that circumstances make it so difficult for me to join you at Madison on Thursday. I would like very much to hear what Daniels picked up from Brackett. However, perhaps we can get together in Chicago, and you can tell us what Daniels found out. Eugene [Rabinowitch] says he could be in Chicago next Monday, May 14th. That would suit me very well. [...] Can you drop me a postal card confirming this plan? If you agree, then I will show up at your place 10:30 Monday morning.[152]

Short as it is, this letter alludes to the following events: a telephone conversation between Emerson, Gaffron, Franck and, presumably, Rabinowitch; a report by Emerson's Urbana colleague Sol Spiegelman, who had seen Burk at a conference in Cleveland; a meeting scheduled at Daniels's laboratory at Madison, at which Daniels would report about an earlier meeting with the expert in polarography and spectroscopy Frederick S. Brackett; and a future meeting in Chicago, at which Gaffron was requested to pass on to Rabinowitch and Emerson the news received from Daniels. One would be entirely justified to see this as reflecting an intense flow of information within certain networks of actors.

The common goal of most of these actors was to explain, if at all possible, how Warburg and Burk obtained their implausible data. This was the main reason why researchers either wanted to go and look at Burk's experiments in Bethesda (Warburg's experiments in Dahlem were too far off for a short visit) or wished to meet independently, in order to discuss experimental methods, data and alternative interpretations. Frequently, both approaches were combined: for example, Kok, as mentioned earlier, went on to Chicago to discuss his experiences at Bethesda with Franck and Gaffron. In a similar vein, Allan Brown visited Burk in Bethesda, and although he was still unconvinced of the validity of Burk's data and his interpretations, he turned to Emerson afterwards with the following inquiry:

> I should like to ask you again about the extent to which you have studied the alleged "accelerated combustion" phenomena. [...] It seems that the situation is in this case different from that of the four quanta dispute. In the earlier controversy Warburg's results could be repeated if one designed the same inherent errors into the experiment, but for the "accelerated combustion" I believe no such duplication has been obtained. Is my interpretation correct that you have looked for the effect and not found it? In discussions with Burk and people of that school it is not very effective to claim that the effect is not observed. Burk counters with the argument that the conditions were not right.[153]

This, of course, hit the nail on the head. The quantum yield experiments had shown themselves to be so complicated that one had truly to master the methods if one were to obtain any useful data at all. Warburg and Burk, like everybody else, were fully aware of the situation and did not hesitate to capitalise on it. And while

[152] Emerson to Gaffron, 8 May 1951, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Gaffron, Hans, University of Illinois Archives.

[153] Brown to Emerson, 25 May 1952, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Brown, Allan, University of Illinois Archives. "Accelerated combustion" refers here to the high oxygen consumption that Warburg and Burk claimed to have found, which was thought to be used for respiratory purposes (that is, for the "combustion" of carbohydrates).

Emerson, in principle, knew what he had to do, in order to undermine this most recent Warburg–Burk approach, he had not yet succeeded in doing so. Emerson's own major criticism of Warburg and Burk's one-minute interval measurements was the following:

> They have no proper basis for justifying the baseline rate of exchange, which would be respiration if they alternated measured light with darkness, but which is an unknown mixture of respiration and photosynthesis in their experiments with unmeasured auxiliary light. The effect of the measured light can only be calculated from some assumed background rate whether or not auxiliary light is used, and I doubt that the provisions they have made for establishing the background rate in their prolonged one-minute-light, one-minute-dark experiments can be regarded as dependable.[154]

Although this was a serious point of concern, Emerson as well as Brown knew that this problem could not possibly be resolved at the moment, either by Burk or by Emerson, and surely not through using of manometric methods. This, of course, was all grist to the mill for Brown, who, at the time, was writing a review of photosynthesis research with Albert Frenkel. Therein, Brown and Frenkel strongly suggested that, in view of the clear limitations of manometry, other methods should be used to settle the debate, preferably physical techniques, such as infra-red spectrometry, polarography, mass spectrometry and others.[155] Emerson, however, never lost his faith in manometry; he continuously strove to improve the technique, for example, by using double differential manometers, which were far more precise than the usual single ones.

In the summer of 1952, preparations got underway for a large conference on photosynthesis that would take place in Gatlinburg, Tennessee, at the end of October, supported by the National Science Foundation, the Office for Naval Research and the Atomic Energy Commission.[156] On 1 July, Emerson received a letter from Hendricks inviting him to participate in this conference, "for the purpose of examining those aspects of the subject which appear to be limiting further understanding".[157] The format was intended to encourage free discussion among the participants: no formal papers were scheduled, but sessions of full half days were reserved for every theme, with an additional, uncommitted day at the end of the conference. An introductory speaker would, at the beginning of each session, briefly review the subject matter; while immediately afterwards the floor would be given to anyone in the audience: "Everyone should be prepared with slides and illustrative material on whatever is felt to be pertinent to the subject," Hendricks pointed out in his letter.[158]

Emerson was feeling pretty desperate when he received the invitation. As he wrote to Charles Whittingham on 5 September, for the greater part of the year he

[154] Emerson to Brown, 28 May 1952, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Brown, Allan, University of Illinois Archives.

[155] See Brown & Frenkel (1953), p. 426.

[156] Hendricks (1953) provides a short summary of the conference's discussions.

[157] Hendricks to Emerson, 1 July 1952, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Gatlinburg Conference, University of Illinois Archives.

[158] Hendricks to Emerson, 1 July 1952, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Gatlinburg Conference, University of Illinois Archives.

had been trying to free himself from Warburg's shadow, leaving the quantum yield question aside and following up some other lines of research, among other things, a thorough study of the carbon dioxide burst and its relationship to temperature and other variables. Emerson was aware that these studies would not contribute to resolving the controversy, yet, as he wrote with some exasperation: "I feel I cannot always submit to being led around by the nose by that deceitful old poker player, and must sometimes test out one or two of my own ideas."[159] However, one full session was being reserved for the quantum requirement question at the Gatlinburg conference, and Emerson knew that, because of Warburg's latest publications, the audience would expect much of his contribution:

> Warburg's new papers, reporting high efficiencies in carbonate mixtures, etc., have excited a good deal of interest. I've tried to give them the brush-off, saying that he has not established the dark-rate on the basis of which he calculates light action, and looking always for the weak spot which I feel sure is there, concealed as cleverly as possible by the crafty old poker player. But our analysis of the errors inherent in Warburg and Burk's 2-vessel technique, adequate though it was for the refutation of their claims up to 1950 or so, is of no help in elucidating the meaning of the one-vessel measurements in carbonate buffer [solution].
>
> Shimpe assures me that at Gatlinburg, everyone will want to know what, if anything, is wrong with the carbonate mixture single-vessel quantum yield measurements. He thinks the trouble comes from Warburg's (obviously incorrect) assumption that there is no significant physical lag, but neither Shimpe nor I can see how this could produce the high efficiencies.[160]

As tired as Emerson was of the whole affair, Burk, who had also been invited to Gatlinburg, was enthusiastic and motivated. Warburg declined his invitation, although he did send Burk some slides with new data to use in the discussion. Burk made diligent preparations, since he did not expect the "game" to be easy. As he wrote to Warburg on 28 August 1952: "I too agree that the cold war on the quantum yield is in fact getting more intense and may soon develop into a hot one, abroad as well as here."[161] Warburg was obviously concerned about the outcome (perhaps he recalled the Sheffield meeting, where Burk did not make a very favourable impression upon the audience) and raised the possibility that Burk should also turn down the invitation. However, Burk had sufficient confidence in himself and thought it would be unwise not to go: "Because if none of us shows up there people will surely get, or maliciously create, the impression that we have 'lost heart' or become afraid." Warburg's alternative suggestion – to find again an impartial judge to pass an authoritative sentence after having heard the arguments – was countered by Burk with the objection that he could not think of anybody who would fit the role.[162] Warburg's concerns that the other side's preparations would be at least as thorough as Burk's were not unfounded. On 27 October 1952, Burk wrote to Warburg, just before he set off for Tennessee:

[159] Emerson to Whittingham, 5 Sept. 1952, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Whittingham, Charles, University of Illinois Archives.
[160] Emerson to Whittingham, 5 Sept. 1952, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Whittingham, Charles, University of Illinois Archives.
[161] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 28 August 1952.
[162] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 12 September 1952.

> A week ago, I am reliably told, Gaffron telephoned here to Brackett and arranged for the extra Saturday session on the quantum requirement, in which Brackett was asked to take the role of ringleader, and Hendricks has warned me: "They are all against you." But we think we are ready for them, and I enclose one of the slides of data which shows not only the 1-quantum measurements in alkaline solution (bottom part of the table) but also how the rate of shaking (if inadequate) can affect the quantum yield (utilization of light) even though the manometric gas equilibration is perfect, as shown by identical rates of respiration at both rates of shaking (150 and 210).[163]

Thus, at the Gatlinburg conference there was to be not one but two sessions on the question of quantum requirements; and one can assume that this was done with the hope of settling the issue once and for all. How the meeting proceeded can be taken both from Burk's elaborate and highly detailed letter to Warburg and from a short review written by Hendricks for publication in *Science*.[164] By all accounts, it was an extremely lively meeting, with discussions lasting each day from early morning till late at night; and the sessions on quantum yields were among the liveliest. The first session, Burk reported to Warburg, was dominated by "the Old Guard", or, as Burk alternatively put it, the "Murderers' Row", which comprised "Brackett, Gaffron, Daniels, Arnold, Emerson, Brown, French, and Franck, etc."[165] Burk continued:

> [They got up one after the other and] beat unmercifully, and surely unscientifically, at both the 4- and the 1-quantum. The attack was far worse than at Sheffield and carefully timed and planned beforehand, to create the impression among the rest of the audience that both the 4- and the 1-quantum values were impossible, absurd and easy to explain. During all this while I didn't say anything but just sat looking unconcerned, smoking one cigar after another, while the situation seemingly got blacker and blacker.[166]

Things were not looking good for Burk's camp. Brackett presented the data that he had obtained using polarographic methods, which showed that the minimum quantum requirements were six to ten per molecule of oxygen. Brown reported that mass spectroscopy gave no evidence of a substantial consumption of oxygen in the dark phases, as would be expected with the one-quantum mechanism. Gaffron demonstrated that photosynthesis could be initiated without any trace of oxygen, that is, without the probability of back reactions occurring such as those described in the one-quantum mechanism. According to Burk, Daniels presented, "with obvious facetiousness (and with some amusement)", the results obtained by Burk's student converts in Daniels's laboratory, one of which had arrived at values of around nine, while the other had "agreed on the average with those of Warburg and Burk but individually varied from 3 to 14, in such manner that no confidence could be placed in them". Finally, Emerson reported that he had been unable to find any difference in outcome between the old and new carbonate mixtures, both of which generally gave quantum requirements of nine.[167] He also

[163] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 27 Oct. 1952.
[164] Hendricks (1953).
[165] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 13 Nov. 1952.
[166] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 13 Nov. 1952.
[167] All quotes: Archive of the BBAW, NL Warburg 174. Burk to Warburg, 13 Nov. 1952.

made it very clear that, in view of the wealth of data that contradicted the high Warburgian yield, it was no longer Emerson's responsibility to explain why he did not get the same results as Warburg and Burk, but that it was now up to Warburg and Burk to examine their experiments to find out why they did not get the same results as everyone else.[168] Emerson most probably made this remark in response to Burk's attitude at this session. As Brown had foreseen in his letter to Emerson, Burk refused to take any notice of other people's measurements, unless they had demonstrated manometrically that they had been able to get a quantum requirement of four under conditions as they had been recently specified by Warburg and Burk. Failures to do so were regarded by Burk as evidence of nothing but the researcher's incompetence.

The discussion was resumed on the last day of the conference, in a session chaired by French. On this occasion, Franck took the opportunity to talk for nearly one hour, explaining in detail the principles underlying the energy accumulation in photosynthesis. This talk made an enormous impression upon the audience. Emerson wrote to Franck afterwards that he had never before heard Franck give so clear an exposition of the energy losses and energy requirements involved in photosynthesis.[169] Even Burk admitted to Warburg that these thermodynamical considerations seemed to be, at present, the most potent objection to the 4- and 1-quantum theory: "Franck looks at this slide [on which the energy balance was written in detail], and then sings a chorus, in which a great number join, that Burk and Warburg are tampering with not only the first but the second law of thermodynamics, in short, denying God himself."[170] In addition to that, Franck also made a new attempt to reconcile the Warburg–Burk data with the data obtained by other groups, along the same lines as earlier (thereby suggesting that Warburg and Burk had, in actual fact, measured the yield of a different photochemical process). This was endorsed by Kok, who was the next to speak and who pointed out that he had witnessed the low quantum requirements being reached only under conditions where respiration was likely to interfere substantially with photosynthesis (compare this with Burk's description of Kok's attitude after his visit to Bethesda). Finally, it was Burk's turn to talk, as he wrote to Warburg:

> [...] I then got up and spoke for the rest of the session, and had the last word, so to speak, or at least the next to the last word, since there was nearly a half hour of discussion after I got through. [...] My general attitude was that here were the data, and our conclusions, but anybody who wished to believe otherwise, for the next five or ten years at least, could do so and see where such other beliefs might lead him.[171]

The general impression one gets from Burk's letter is that he had finally lost all sense of reality. Burk continuously divided people into two camps: the supporters of his and Warburg's "cause" on the one side; the opponents on the other. Burk again emphasised that at Gatlinburg he had also managed to "get a number of

[168] Archive of the BBAW, NL Warburg 819. Arthur Schade to Warburg, 5 Dec. 1952.
[169] Emerson to Franck, 13 Nov. 1952, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Franck, James, University of Illinois Archives.
[170] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 13 Nov. 1952.
[171] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 13 Nov. 1952.

new converts to our side", while he frowned at "a middle group of fence-sitters, like Sterling Hendricks, who still mainly point their heads in the direction of the other side and their tails to ours"; to which Burk added: "In any dictionary of the American language, such people are known as mugwumps."[172] Obviously, Burk found this refusal to take a stand completely unacceptable.

It also seems that people at the conference, notably Hill, had the impression that Burk was on a slippery slope and that it would be wise if he detached himself from Warburg before it was too late – the sooner, the better. Burk reported these details to his master:

> Well, in addition to science, there were all kinds of personalia and various "kind" people like Robin Hill and Kok and others advised me to drop any further work on the quantum yield or even photosynthesis in general before I lost my scientific reputation altogether! That you should lose yours was perhaps of lesser moment to them, than that such a nice and kind person as myself should do so!!! And so the crocodile tears rolled on, from some people. On the other hand, after the final meeting, Calvin, who I am sure is no great believer in the 4- and 1-quantum, told my wife, "You tell your husband to keep on fighting."
>
> Robin Hill, who is the only person to flatly refuse to contribute to the festschrift [in honour of Warburg's seventieth birthday], spoke very frankly to me and it is quite obvious that he shares the Cambridge views about you personally, and he said to me quite frankly that in his opinion you were a "rogue" and did not mind who knew it. He also stated to me that he had the highest opinion of Emerson personally and scientifically, and it is obvious that they are great friends [...]. Hill said that you have been unforgivably rude to not only Keilin but various others at Cambridge. I asked him whether it was more important to be rude or actually scientifically wrong and stupid, and he said frankly that he preferred to be stupid rather than rude – a matter of taste in any event.[173]

The interesting point here, however, is that, although the majority of photosynthesis researchers now believed that Burk and Warburg were using dirty rhetorical tricks and that they were behaving in an utterly unacceptable manner when it came to dealing with factual criticism and divergent points of view, this did not change the fact that the value of the photosynthesis quantum yield was still unknown. So, for example, even though Hill was thoroughly disgusted by Warburg and Burk's conduct, this did not prevent him from paying a visit to Burk's laboratory shortly after the conference, in order to see the disputed results with his own eyes and to discuss photosynthetic matters with Burk. Indeed, Hill had tried, together with Whittingham, to reproduce the Warburg–Burk values with his haemoglobin method; the attempt had failed, but Hill wished to try it again and therefore needed to go over some of the experimental details, as Burk proudly explained to Warburg: "He [Hill] agreed that just plain negative results, without understanding, mean nothing, and he agreed that Brown's results wouldn't mean anything until Brown first repeated our results and could then show, by the mass spectrograph, if anything were wrong with them."[174]

[172] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 13 Nov. 1952.
[173] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 13 Nov. 1952.
[174] Archive of the BBAW, NL Warburg 174. Burk to Warburg, 13 Dec. 1952.

In truth, Hill was very interested in how respiratory back reactions might interfere with photosynthesis and its quantum yield; and this interest was clearly strong enough for him to try and find out from Burk as much as possible about the measurements in question – even though, on a personal level, Hill would have preferred to talk to somebody else. The new measurements taken in a carbonate buffer solution really had reintroduced a new sense of uncertainty. While the carbon dioxide burst had undermined all the measurements taken in an acidic medium, Warburg and Burk now seemed to be able to measure low quantum yields in carbonate solutions. The fact that Emerson claimed to be unable to reproduce the data was not received as a satisfactory counter argument, and justifiably so. Negative results – in this case, the failure to replicate a certain experimental finding – were not a fatal blow, particularly when the experiments required such delicate handling, as quantum yield measurements did. Whittingham succinctly summarised the situation, as he and Hill saw it, in a letter to Emerson in March 1953:

> The question arises – supposing W's observations were genuine – what possible explanation is there? One postulates a further carboxylation process in the stronger buffer. One then tries to find evidence for or against that point of view. This we think is found lacking [...]. The isolated fact that W. did or did not get what he claims is of little interest without reference as to how it affects one's preconceived notions. So we think![175]

Interestingly, Burk received the 1952 Hillebrand Prize of the Chemical Society of Washington (the local American Chemical Society Chapter for the Washington, DC area) "for the experimental discovery of a photosynthetic energy cycle of high quantum efficiency, with demonstration of the applicability of the Einstein law of photochemical equivalence".[176] Emerson was well aware that, because of Warburg's carbonate data, he now had to defend his position once again; and he was also aware that he and his colleagues were engaged in battle with a powerful enemy, in both scientific and rhetorical terms. After the conference, Emerson wrote to Franck :

> This letter is primarily to express to you my appreciation of your presence among those of us who are working in the field of photosynthesis. You have sometimes been distressed because you felt your contribution was not as great as you would like to make it. But as I listened to you at Gatlinburg I felt, more than I ever did before, the value of the leadership which you have brought to the field. Your presence among us was an incentive to all of us to make our own contributions on the highest possible plane. There are not many people who could provide this sort of inspiration, and you are the only one in the photosynthesis group. (I must say that I think Hill may come in time to exert a similar quality of leadership, though in quite a different way, because he lacks your background in physics and photochemistry.) I need not mention to you the names of the other men who would be dominant figures in the photosynthesis group if you were not among us, but their names and faces

[175] Emerson to Whittingham, 2 March 1953, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Whittingham, Charles, University of Illinois Archives.
[176] Archive of the BBAW, NL Warburg 174 Burk to Warburg, 17 Jan. 1953.

> flitted through my mind as I listened to the talks in Gatlinburg, and I thought how glad I was that you were with us.[177]

One of the more practical outcomes of the meeting was that the two groups from Urbana and from Chicago strengthened their ties: regular trips were made to the other group's colloquia and graduate students were encouraged to follow up the other group's activities and findings. Joining forces certainly seemed to be the best strategy in view of the precarious situation.

7.4 Emerson Strikes Back (1955)

Even though Emerson was weary of the maximum quantum yield question, after 1952 he continued to work relentlessly towards obtaining results that would validate his own point of view and refute the Warburg–Burk picture of photosynthesis. In January 1954, Emerson apologised to Hill for having failed to keep in touch with him – the reason being, Emerson explained, that he had slowly started to obtain some useful experimental results:

> It was a matter of achieving a combination of very improbable states, simultaneously. Enough light energy, necessary optical parts, cathetometer telescopes, Mrs. Chalmers getting enough experience in taking readings, etc., etc. We are beginning to find out how Warburg and Burk can get *some* of the results they claim. After several years of deeply disappointing and frustrating failures, when I suddenly began to get some hopeful results, I just decided to neglect everything else. Even so, the work seems to move at a snail's pace. The cellular processes are terribly intricate, that is to say, the cells have so great a capacity for adjustment that no single experiment is ever by itself conclusive. Each day's work seems to require that 10 more days be spent to clear up the new doubts raised. But at least I am working on the cells and their photosynthesis, and not on the apparatus![178]

Emerson spent much of 1954 with Ruth Chalmers, a long-standing co-worker and expert in algae culturing, on a sabbatical leave in Briggs's laboratory at the University of Cambridge. There Emerson found the necessary peace and freedom to focus on the required experimental work and on writing it up. The result was a lengthy manuscript, co-authored with Chalmers, which was completed in May 1955. Emerson submitted the paper to the journal *Plant Physiology* and, at the same time, sent out copies to a number of colleagues, whom he asked for comments. In the accompanying letter to Daniels, Emerson explained why the text had grown so much in length:

> I feel apologetic about the length of the manuscript, but it is a good deal shorter than the sum total of the papers Warburg and Burk have published during the time we spent doing this work. It was my hope that I could write something which would provide readers with a basis for forming an independent opinion on the significance of the Warburg–Burk contributions, and save them the embarrassment of basing their opinions on the personal

[177] Emerson to Franck, 13 Nov. 1952, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Franck, James, University of Illinois Archives.

[178] Cambridge University Library, Ms. Add. 9267/J.54, Emerson to Hill, 4 Jan. 1954.

> prestige of the authors. In my efforts to achieve this, I'm afraid, I let the paper become much too long![179]

Franck was the next to receive a copy of the text. Emerson also requested a very specific type of comment from him:

> I would like very much to know whether you think the standpoint from which it is written is a useful one, and also whether you think I have suppressed contentious remarks about Warburg and Burk. I wish I could make my writing as free of prickly statements as yours is. [...] I'm sorry that it is so long. Maybe I'm beating a dead horse?[180]

The manuscript succeeded in making a major impression, as one can take from a letter Emerson wrote to Whittingham shortly thereafter: "I have a long letter from Gaffron with his comments, and have spoken with Franck on the telephone about it. Gaffron tells me that for the first time Franck begins to understand my objections to the experimental work of Warburg and Burk!"[181] On 28 July, the paper was accepted for publication, although the journal's editor, David Goddard, strongly recommended that either the material should be reorganised or that it be divided into two papers. However, in the end it remained in one piece, published, in November of the same year, as Emerson & Chalmers (1955).

The paper took up no less than twenty-six pages of the journal, and revisited all the major criticisms directed at their experiments and diverging points of view. The main purpose of the paper was, as Emerson and Chalmers emphasised, to consider the value of the methods with which photosynthetic efficiencies of 70 per cent or even higher had been found. Their two primary concerns in doing so was: first, to establish the existence or non-existence of a time lag between the changes of gas pressure inside the cell and the observable changes in the manometer readings; and, second, to establish the potential influence of transient gas exchanges on the calculations of photosynthetic efficiency. Revisiting this question, Emerson and Chalmers underlined, had become necessary in view of Warburg's and his co-workers' insistent claim that time lags between the changes in rates did not influence their data, although they had taken readings at intervals that had sometimes been as short as one minute, without having made any allowances for a time lag. Emerson and Chalmers gave a detailed evaluation of the methods involved, and drew the following clear-cut conclusions:

(i) Even under the conditions chosen by Warburg and Burk the influence of time lag was appreciable, primarily due to diffusion effects between liquid and gas space.

(ii) Apparent absence of time lag was due to the obscuring effect of compensatory processes in the vessel.

[179] Emerson to Daniels, 16 June 1955, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Daniels, Farrington, University of Illinois Archives.

[180] Emerson to Franck, 17 June 1955, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Franck, James, University of Illinois Archives.

[181] Emerson to Whittingham, 25 June 1955, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Whittingham, Charles, University of Illinois Archives.

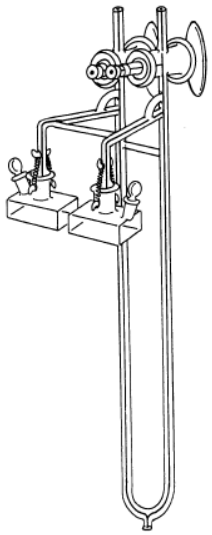

Figure IV.10: Differential manometer: "One vessel is used as a compensating vessel with suspending fluid but no cells. The other vessel is for the experimental material. For two-vessel measurements, a second manometer is required, with a pair of vessels larger than the pair shown here." The advantage of this type of manometer is that it is a closed system that requires no additional barometer vessel and no pinchcock adjustment for constant volume. Reproduced from Emerson & Chalmers (1955), p. 507.

If these factors were not taken into consideration when interpreting the manometrically obtained quantum yield data, the efficiency calculations would be affected in a complicated, and surely significant, manner.

In order to establish these claims, Emerson and Chalmers had striven to duplicate exactly the Warburg–Burk experimental set-up, even though they found it inadequate in many respects. They diverged from this set-up in only one respect, namely, in their choice of manometer. In explaining their reasons for this, Emerson and Chalmers drew attention to the fact that, if they had used Warburg's two-vessel method, then they would have needed to take three manometer readings while the manometer was being vigorously shaken. "Even with the aid of a hand lens, a precision of $\pm$ 0.5 mm is the utmost that can be expected," Emerson and Chalmers maintained; as Warburg's findings were often taken from pressure changes of 3 mm, the resulting range of uncertainty amounted to about 30 per cent![182] "Greater precision is attainable," Emerson and Chalmers explained, "by reading the manometers with a cathetometer (horizontal telemicroscope with cross-

[182]It is interesting to see that, besides the paper he wrote with Nishimura and Whittingham, Emerson also cited the work carried out in the laboratory of the German plant physiologist André Pirson, namely Pirson, Krollpfeiffer & Schaefer (1953). Pirson was one of the few experts working

hairs and screw adjustment for height, and scale divided into hundredths of a mm), but the constant-volume type of manometer [which Warburg used] does not lend itself to reading by cathetometer". Therefore, in contrast to Warburg's protocol, the authors used differential manometers, which would enable them to reduce reading errors to a precision of $\pm$ 0.03 mm. With the help of these instruments, Emerson and Chalmers demonstrated that, under the conditions chosen by Warburg and using his methods of calculation, enormously high quantum yields could be reached, although they did not reflect the quantum yield of photosynthesis. The authors painstakingly spelled out the details of their set-up, mentioning every possible source of error in the experiments (and, at the same time, demonstrating that their errors were much smaller than the ones implied in Warburg and Burk's experiments); they were able to explain how the changes Warburg made to his set-up altered some of the details of his data; they demonstrated that a diffusion lag was a factor that, even under the conditions chosen by Warburg, influenced the outcome of the experiments in relevant ways (although Warburg constantly claimed otherwise); and they concluded that, on every account, the application of the two-vessel method, constantly used by Warburg and his co-workers, was inappropriate for determining transient metabolic rates. This is how Emerson and Chalmers formulated the essence of the paper:

> This discussion of the interrelations of diffusion lag and transitional rates of gas exchange leads inevitably to the conclusion that measurements of the efficiency of photosynthesis are significant only when they are based upon steady metabolic rates. The results we have reported here support the conclusion reached earlier by a number of other investigators, that a quantum requirement of about eight per molecule of oxygen produced represents the highest efficiency that can be sustained by the evidence (equivalent to about 30% in red light). The claims put forward by Warburg and co-workers that from one to four quanta suffice per molecule of oxygen produced, appear to be founded upon experimental methods which cannot be counted upon to give results which are numerically correct, and the results, whether correct or not, cannot be regarded as an appropriate basis for calculating the efficiency of photosynthesis.[183]

As a postscript to this, Warburg had, in the meantime, turned to S. D. Cornell, the Executive Officer of the National Academy of Sciences (NAS) of the United States. In his letter, dated 25 July 1955, Warburg had expressed his concern that several important developments in the field of photosynthesis research, achieved in his laboratory, were continuously being contested by American scientists, notwithstanding the fact that these achievements had completely changed the general understanding of photosynthesis. Warburg reminded Cornell of the fact that "in the days of Pasteur, whose discoveries were often contested in a similar way, the French Academy of Sciences settled the disputes by naming commissions to look at and to check the disputed experiments".[184] He therefore appealed to Cornell

in Germany at the time who was highly critical of Warburg's claims. See Pirson (1994) for an autobiographical review of Pirson's work.

[183] Emerson & Chalmers (1955), p. 528.

[184] A copy of Warburg's letter is preserved in: Franck, James. Papers, [Box 10, Folder 1], Special Collections Research Center, University of Chicago Library.

to send such a commission to Berlin-Dahlem, where Warburg had recently set-up a special laboratory for demonstration purposes. Cornell forwarded this letter to Calvin, Daniels, Emerson, Franck, Goddard and Hendricks, as the experts among the Academy's members, with a request for suggestions as to how the Academy should respond.

The answers Cornell received were clear and unanimous. Emerson wrote that he did not see how sending a delegation to Berlin would serve any good purpose. He reminded Cornell that, for a long time, a number of courteous efforts had been made to draw Warburg's attention to errors that were inherent in his measurements, but none of these had elicited from Warburg any sign of willingness to re-examine his work. "Rather, he has shown an arrogance in overlooking [his critics'] work and writings, which is unbecoming to his profession. Sometimes he has evaded criticism by cunning and specious arguments," Emerson wrote. A visit by a delegation of the Academy, Emerson believed, would not help to clarify the question, "but would probably be made the basis of a new emphasis upon his prestige, a circumstance which is without direct bearing upon the problems of photosynthesis with which we are concerned".[185]

Franck's response to Cornell was that Warburg's letter placed him in an awkward position; if Warburg received a negative answer, he was likely to use this as an argument for the fact that his opponents shunned the objective testing of results. Yet Franck could still not agree to setting up such a committee. First, he underlined that it was impossible to settle the dispute this way: "Apparently Warburg supposes that it is enough to demonstrate a few examples of manometric measurements from which data may be calculated which support his views. If most of the data do not give the desired results he will explain as he has done often that it is only necessary that some of the data fit because one cannot expect that the biological material is always present in perfect conditions." However, the main reason that Franck advised against sending a delegation was more fundamental and was based on his understanding as to how science should be made to work:

> I believe that it is not the task of our academy to sit in judgment about scientific differences of opinions. [...] [T]he decision what is right and what is wrong should be left to the normal process of the development of science which is after all, a very efficient way to weed out errors even if the processes might not be as quick. After studying the problem carefully for years, I am convinced that the right is not on Warburg's side. Even a scientist as outstanding as Warburg can be occasionally wrong and that is to my regret this time the case in his photosynthetic studies of the last years.[186]

This was also Calvin's opinion: the question whether or not an individual's results and interpretations were accepted by others should be determined in the usual way, Calvin wrote to Cornell, "namely, by the willingness and interest of the scientific world in the form of the collection of individual scientists to undertake

[185] Emerson to Cornell, 10 Aug. 1955. Franck, James. Papers, [Box 10, Folder 1], Special Collections Research Center, University of Chicago Library.

[186] Franck to Cornell, 15 Aug. 1955. Franck, James. Papers, [Box 10, Folder 1], Special Collections Research Center, University of Chicago Library.

to test the results and theories proposed by Prof. Warburg".[187] Finally, Daniels informed Emerson that he had discussed the subject in informal talks at a conference in Geneva, Switzerland, with Rabinowitch, Calvin and the physiologist Detlev W. Bronk, who was at the time President of the Academy. All four of them had agreed that "it would be a bad precedent for the NAS (already mentioned) to appoint a committee when scientists disagree. There would be no end of such committees." To this more official decision, Daniels added in his letter to Emerson: "Personally, I do not feel that Warburg is entitled to any more consideration than was given to him by you and your laboratory a few years ago. Warburg's letter is really quite astounding. The less attention we pay to it, the better."[188]

8 The Aftermath

8.1 The Enhancement Effect

By Emerson and Chalmer's 1955 paper, most people in the field had become convinced that the numbers of eight to ten quanta as a minimum requirement of photosynthesis was at least approximately the accurate one; while any further (and, perhaps, a more definitive) resolution of the question had to wait for the development of new methods. A general saturation point had been reached and many participants felt that the problem had been discussed for far too many years and in far too much depth – almost *ad nauseam*. This was reflected, for example, in the fact that the theme of the maximum quantum yields of photosynthesis was deliberately excluded from the Second Gatlinburg Conference on photosynthesis, held in October 1955.[189] Not that Warburg had stopped publishing ever new variations of his experimental set-up, which always gave the same high quantum yields. In reaction to Warburg's most recent papers, Gaffron wrote the following letter to Burk in March 1956:

> Dear Dean
>
> I delayed this note of thanks until we had sent you our recent papers in reciprocation of your kindness in forwarding us the latest reprints from Warburg's laboratory. These publications on photosynthesis have now clarified the situation rather definitely: "Too strong tobacco to smoke in my Meershaum!" [sic] The deviations from the experiments and theories of other workers in the field are wonderfully clear. Soon there will be no need to concern oneself with the matter any further.
>
> What I am wondering is to what extent you personally are willing to believe in and subscribe to what comes from Dahlem? For us it would simplify the situation if we were allowed to identify you entirely with the Warburg school, but I cannot help feeling that this might do you a serious injustice. You should feel young enough to dare to deviate from the party doctrine the moment you recognize how absurd the tenets are in which followers are asked to believe. Has that moment arrived?

[187] Calvin to Cornell, 26 Aug. 1955. Franck, James. Papers, [Box 10, Folder 1], Special Collections Research Center, University of Chicago Library.

[188] Daniels to Emerson, 26 Aug. 1955, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Daniels, Farrington, University of Illinois Archives.

[189] The papers of this conference were published in the volume Gaffron, Brown, Stacy, Livingston, Rabinowitch, Strehler & Tolbert (1957).

Very sincerely yours, Hans G. Gaffron.[190]

Gaffron's disbelief and consternation concerned, among other things, Warburg's claim (first published at the end of 1954) that, in order to compensate for respiration, no high intensity white background light was necessary, as he had believed up to then, but that blue or green light beams of rather small intensity were sufficient, the effect of which Warburg called "catalytic".[191] Despite the fact that many of the players had reached saturation point, this paper was nevertheless broadly discussed. Thus, the Stanford-based plant physiologist Lawrence Blinks wrote to Emerson on 28 September 1955: "What do you think of Warburg and Krippahl's strange findings on blue light (after red)? I've never tried exactly that experiment, but I now aim to, on *Ulva* or *Monostroma*. Off hand, I don't believe it, but strange things do happen."[192] Emerson replied to him on 10 October:

> As for Warburg's blue light experiments, the wave length is about the same as that in which Tony Lewis and I found evidence of strong effects of light on respiration. I've tried to think of an interpretation that would account for both our observations and Warburg's, but they don't seem to agree. Warburg's manometry has become almost mystical, and I'm generally pretty skeptical of his interpretations, but I do believe there are some special effects of certain wave lengths of blue light. I'll be interested to hear what you find with *Ulva* and *Monostroma*. We hope to do some experiments with *Chlorella* and *Porphyridium*, as soon as we are satisfied with our measurements of light absorption.[193]

The experiments on the effect of combining lights of different colours foreshadowed a fruitful line of research that Emerson pursued in the second half of the 1950s. In December 1955, Emerson wrote to Hill to inform him that he was now working again on photosynthesis efficiencies at different wavelengths, "a subject that warms my enthusiasm quite a bit more than the problems of two-vessel manometry. Maybe we shall come to trying some mixtures of blue and red light, though when one looks closely at Warburg's data, it seems that there is no clear evidence for the special effect of blue."[194] One can take from his correspondence that Emerson did indeed take up this line, which led him to make an unexpected discovery. In a letter to Arnold on 9 April 1956, Emerson wrote: "The significance of the long-wave limit is becoming very interesting. Accessory illumination with shorter wave lengths makes the increment of photosynthesis attainable with very long wave lengths higher than it is without accessory illumination."[195] Emerson wrote to Franck a

[190] Archive of the BBAW, NL Warburg 174. Gaffron to Burk, 7 March 1956.

[191] Warburg, Krippahl & Schröder (1954). See also Warburg, Krippahl & Schröder (1955) for extensions of this new idea.

[192] Blinks to Emerson, 28 Sept. 1955, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Blinks, Haxo, University of Illinois Archives. *Ulva* and *Monostroma* are genera of (multicellular) green algae.

[193] Emerson to Blinks, 10 Oct. 1955, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Blinks, Haxo, University of Illinois Archives.

[194] Cambridge University Library, Ms. Add. 9267/J.54. Emerson to Hill, Dec. 26, 1955.

[195] Emerson to Arnold, 9 April 1956, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Arnold, William, University of Illinois Archives.

week later, informing him of the same information as well as adding the following assertion:

> The amounts of accessory light required are considerable – larger than the amounts of the red beams being used. "Catalytic" amounts of accessory light are not sufficient. The accessory light may be red, green, or blue. We do not yet know what would be the effect of large intensities of the region 670-700 mμ, because we have no suitable filters for isolating an accessory beam of this range. We have ordered some filters, but I am not sure they will give us enough energy. It is difficult to isolate this region without contamination of shorter wave lengths.
>
> This letter should reach you by Wednesday. I plan to telephone you Thursday morning at about 10 o'clock. Please excuse the brevity of this rather hasty letter. I should be setting up an experiment.[196]

Emerson was first clearly excited about his findings and urgently requested Franck's opinion and advice; and, second, anxious, already then, to differentiate between his findings and Warburg's catalytic effect of blue and green light. Emerson presented a preliminary account of his work to the annual meeting of the NAS, between 23 to 25 April 1956. First, he noted that at higher temperatures photosynthetic efficiency dropped at shorter wavelengths. This was a blow to the assumption that the drop in photosynthetic efficiency at low-intensity light of the far red was due to the fact that the light quanta of this region had insufficient energy. In this case, the increase in temperature should have improved the quantum yield – yet the opposite was the case. The second surprising discovery reported by Emerson was the following: "If the low-intensity light beam of measured energy is supplemented by a more intense (unmeasured) beam, then the efficiency of the small increment of measured light remains nearly constant out to 685 mμ, even at a temperature of 26°C."[197]

By November, Emerson had put the results of the year's work into manuscript form, which he mailed off to the *Proceedings of the National Academy of Sciences* (PNAS). It was circulated in advance to Gaffron, Arnold, Briggs and Hill, which left Emerson without any more spare copies, although he would also have liked to send one to Blinks for comments (since Blinks had, together with Francis Haxo, also found a long wavelength decline in the photosynthesis efficiency of green and brown algae, which they suggested might be because sections of the chlorophyll might be inactive).[198] In his letter to Blinks, Emerson described the content of his paper as follows:

> I have proposed an interpretation which I think fits your results on red, brown, and green algae, as well as some new observations of ours. I believe my interpretation is the nearest thing to an "idea" that I have ever produced in my life. It is speculative, and Rabinowitch was at first inclined to be very doubtful of its value. Recently he said he thought it the most plausible alternative he

[196] Emerson to Franck, 16 April 1956, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Franck, James, University of Illinois Archives.

[197] See the abstract of the paper, published in *Science* 123 (1956), p. 673, co-authored by Emerson, Chalmers, Carl Cederstrand and Marcia Brody.

[198] Cf. Haxo & Blinks (1950).

> could think of for explaining observations up to the present time, but he hopes that new evidence will suggest other and more acceptable interpretations.
>
> I am suggesting that photosynthesis requires excitation of some pigment in addition to chlorophyll *a*, with an energy level higher than the first excited state of chlorophyll *a*. This would account for the low yield when chlorophyll *a* is the sole light absorber, as far as green algae are concerned. Chlorophyll *b* would be the pigment with a higher excitation level. In brown algae it might be chlorophyll *c* or fucoxanthol. In reds and blue-greens it would be a phycobilin, or combination of phycobilins. It seems to me this promises to account for the observed limits of full photosynthetic efficiency in all cases. Rabinowitch is concerned because it fails to account for the decline in yield of fluorescence on the long-wave side of the chlorophyll *a* absorption band, a phenomenon which he feels should have a common basis with the decline in yield of photosynthesis in the same spectral region.[199]

However sceptical Rabinowitch was about the propounded interpretation, Emerson's paper was published in the PNAS of January 1957 as Emerson, Chalmers & Cederstrand (1957). It had been known since the work done by Emerson and Lewis in 1943 that at low light intensities, photosynthetic efficiency dropped at wavelengths above 685 nm, although chlorophyll *a* absorption was still appreciable; now Emerson had found, with some of his co-workers, that the yield under these conditions could be improved by supplementary light of shorter wavelengths. The limit of wavelengths that were still effective as a supplement was identified to be somewhere between 644 and 680 nm. Considerable emphasis, again, was put on the fact that this phenomenon was very different from the catalytic blue light effect reported by Warburg et al.[200] The tentative explanation given in the paper was the one Emerson had outlined in his letter to Blinks. Emerson, Chalmers and Cederstrand suggested that "the significance of the supplementary light may be that it adds excitation of other pigments besides chlorophyll *a*. The maintenance of maximum efficiency may require the excitation of some pigment with an absorption band corresponding to an energy level higher than the first excited state of chlorophyll *a*."[201] In the green algae this pigment might be chlorophyll *b*. The fact that this interpretation did not conform to other recent notions concerning the energy transfer in photosynthesis was openly acknowledged by the authors:

> [This interpretation] is in conflict with the widely accepted view that transfer of excitation energy to chlorophyll *a* from other pigments takes place with practically 100 per cent efficiency [cited Duysens (1952)]. It also fails to account for the reduced yield of fluorescence on the long-wave side of the absorption band of chlorophyll *a*. However, if further work should confirm our

[199] Emerson to Blinks, 30 Nov. 1956, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Blinks, Haxo, University of Illinois Archives.

[200] This discussion of Warburg's work was critically remarked upon, in advance to publication, by Gaffron, to which Emerson replied: "As for Warburg, I agree with you that our reference to his work with supplementary light will lead to controversy, but I do not feel it would be right for us to report work with supplementary light, without at least a reference to his work. This is the sort of thing he does to us all the time, and the least I can do is set him a good example." Emerson to Gaffron, 14 Dec. 1956, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Gaffron, Hans, University of Illinois Archives.

[201] Emerson et al. (1957), p. 142.

> suggestion that photosynthesis requires excitation of two different pigments, we can hardly expect to find a common interpretation for the long-wave decline in yield of fluorescence and photosynthesis.[202]

Emerson undoubtedly realised that these were not minor points; he would have preferred to come up with an interpretation that gave a common cause for both phenomena: the drop in photosynthesis efficiency and the drop in chlorophyll *a* fluorescence. However, he was unable to find one, as he wrote in a letter to Franck on 22 January 1957.[203] Emerson and his co-workers continued their investigation of the effect. In the first months of 1957, they focused mainly on the influence of intensity and wavelength on the supplementary light; besides his general wish to explore the effect and its causal context in more detail, Emerson was probably still driven by the goal to distinguish his supplementary light effect from Warburg's idea of catalytic blue light effects.[204] This was also emphasised in his subsequent presentations, in 1957 and 1958, at the meetings of the NAS.[205] Under no circumstances did Emerson want to be cited as confirming Warburg's results, although he did acknowledge that he had hit upon the phenomenon in question while double-checking the catalytic light claim. In July 1957, Emerson wrote to Hill: "I've had quite an exciting time with the experiments on mixing long-wave light with shorter wave lengths. The effects do not match Warburg's claims at all, but of course we were stimulated to do the experiments because of Warburg's claims."[206]

8.2 Emerson's Death and Beyond

On 4 February 1959, Emerson died in an aeroplane crash. He had always distrusted aviation as a means of transport, preferring to travel around the country by train. It was only because the train service from Indianapolis to New York had been discontinued in the late 1950s that he had grudgingly turned to flying between Chicago and New York. This particular time, Emerson had wanted to attend a conference at Harvard University. Even more tragic was the fact that Emerson was originally booked on another flight; yet when he arrived at Chicago, a flight that had been delayed was still waiting to depart for New York; at the last minute Emerson transferred to it, hoping that he would arrive at his destination a little earlier. This turned out to be a fatal decision.

Emerson left behind a great deal of experimental material at the Urbana laboratory, accumulated during the years that he had been working on the long-wave limit of photosynthesis; only some of this work was published posthumously, in 1960, by Emerson's friend and Urbana colleague Rabinowitch. It was in this publication that the phenomenon in question was called, for the first time, the

[202] Emerson et al. (1957), p. 142.

[203] Emerson to Franck, 22 Jan. 1957. Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Franck, James, University of Illinois Archives.

[204] In addition to the sources cited earlier, see also the review article Warburg (1958).)

[205] See the abstract of the paper, published in *Science* 125 (1957), p. 746, with Robert Emerson as the sole author, although his collaborative work with Chalmers and Cederstrand was acknowledged. The failure to replicate Warburg's findings was repeated again in Emerson's presentation at the 1958 NAS meeting; see the abstract in *Science* 127 (1958), pp. 1059f. The suggestion that accessory pigments were responsible for the Enhancement Effect was also repeated in Emerson & Chalmers (1958).

[206] Cambridge University Library, Ms. Add. 9267/J.54. Emerson to Hill, 6 July 1957.

"(second) Emerson effect" (while the carbon dioxide burst was named the "first"); the effect that supplementary light of shorter wavelengths is able to make up for the drop in photosynthetic efficiency at longer wavelengths later became known as the "Emerson Enhancement Effect".[207] In this 1960 paper, Rabinowitch duly presented Emerson's data, although he argued against the assumption that the phenomenon was due to a direct contribution of chlorophyll *b* in photosynthesis: Rabinowitch believed that the evidence derived from fluorescence experiments, which demonstrated that a large fraction of the quanta absorbed by chlorophyll *b* was transferred to chlorophyll *a* by resonance, made this highly unlikely. He suggested, as an alternative, that two types of chlorophyll *a* were present in the living cell, one of which resembled the chlorophyll *b* more closely than the other, while the other was unable to sensitise photosynthesis to its maximum quantum yield. The question was pursued further by Emerson's two remaining doctoral students, Govindjee and R. Govindjee, both of whom were involved in projects related to the supplementary light – or enhancement – effect. Together with the biophysicist Jan B. Thomas and Rabinowitch, Rajni Govindjee was able to establish, in a cell-free system, that the Enhancement Effect was a real phenomenon: it was not an artefact; it was not caused by respiration; and it was not associated with the carbon dioxide reduction process.[208] Together with Rabinowitch, Govindjee succeeded in establishing the existence of two forms of chlorophyll *a*, which had distinct functions in the photosynthetic process in *Chlorella* cells.[209] Both arrived at figures of eight to twelve light quanta as a minimum requirement for photosynthesis; this number was reconfirmed in Govindjee, Rabinowitch & Govindjee (1968), when the authors made an ultimate attempt to replicate those conditions and materials that Warburg had specified in his latest papers, that is, the use of blue catalytic light, 10% carbon dioxide and young *Chlorella* cultures.

The curious enhancement phenomenon and the sudden emergence of different forms and functions of chlorophyll triggered a wealth of further investigations in the field of photosynthesis. More and more findings were accumulated, the interpretation of which became the subject of intense debate (see Chapter VI; at the same time, the question of determining maximum quantum yields gradually faded from the scene. While Warburg seemed to believe that Emerson's death had decided the matter in his favour – he was overheard stating this in public[210] – the real reason was that the question had lost its attraction and importance. Far more exciting new developments needed to be clarified, and they were more likely to lead to more immediate advances being made in understanding photosynthesis than the continued pursuit of quantum yield numbers.

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[207] See Emerson & Rabinowitch (1960).

[208] See Govindjee, Thomas & Rabinowitch (1960*b*).

[209] See Govindjee & Rabinowitch (1960) and Rabinowitch & Govindjee (1961). French (1961) (presented in March 1960) had independently come to the same conclusion. See also Chapter VI of this book, section 5.5.

[210] Govindjee (personal communication) was recounted this episode by Rabinowitch, on his return from a conference held at Gif-sur-Yvette (south-west of Paris) in 1963.

9 Concluding Remarks

In Germany the controversy was received differently. One of the reasons was that Warburg was hugely influential in his own country, and this influence declined only gradually. This was primarily due to the fact that he published predominantly in German journals, which in Germany were far more widely read than their American or British counterparts, and also because Warburg was held in such high esteem in Germany: he was without question regarded as the leading authority in the field.[211] However, he received a strong blow to his authority when in 1961 it was not Warburg who received the Nobel Prize for Chemistry, honouring his work in photosynthesis, but rather the US-American chemist Melvin Calvin, that is, one of Warburg's stubborn opponents. In the announcement of the year's Nobel Prizes, in December 1961, the weekly magazine *Spiegel* gave a sarcastic report of the situation.[212] It was described, how in 1957, Warburg had proudly declared in a public lecture that, thanks to his work, Germany had been able to maintain its international leadership in photosynthesis research, despite the Second World War and the country's collapse. The decision of the Nobel Prize Committee to award the Chemistry Prize to Calvin and not Warburg only four years later was felt to be in stark – and disillusioning – contrast to the pompous self-confidence of Warburg's. Now, slowly, Germany began to realise that Warburg, through ignoring the work done in other laboratories, had become more and more isolated within the international scientific community and that, as a consequence, his contributions were increasingly off the mark.

Although Warburg seemed undisturbed by these developments, and would still claim in his publications of 1970 (the year of his death) that he had solved the problem of photosynthesis, the late biochemist Birgit Vennesland recorded and passed on to the public the following remarkable quotation. When Vennesland asked him whether he had made any mistakes in his life, Warburg replied:

> Of course, I have made mistakes – many of them. The only way to avoid making any mistakes is never to do anything at all. My biggest mistake was to get much too much involved in controversy. Never get involved in controversy. It's a waste of time. It isn't that controversy itself is wrong. No, it can be even stimulating. But controversy takes too much time and energy. That's what's wrong about it. I have wasted my time and energy in controversy, when I should have been going on doing new experiments.[213]

[211] The following additional factor should perhaps also be taken into consideration: in the spirit of self-pity prevailing in the postwar years, many Germans (particularly non-experts) were inclined to frame the controversy between Warburg and the USA in political terms, as another aspect of the unjustified repression of Germans by the US "invaders". On the atmosphere among scientists in postwar Germany, see, e.g., Deichmann (2001*a*) (which focuses on biochemists) or Hentschel (2005) (on physicists). Pirson (1994) (p. 215), however, provides a perspective that is more in line with the thinking of American colleagues; he attributes the lack of involvement of the Germans in this controversy to the general inadequacies of the universities' experimental equipment, which simply did not allow German researchers to contribute usefully to this difficult question.

[212] See Anonymous (1961).

[213] Quoted in Govindjee (2004*b*), p. 185.

Since Emerson also felt that he had spent too much time and energy on the quantum yield controversy,[214] as well as almost everyone else working around them, it is surprising that the controversy had not ended much earlier. I shall now summarise the argument again, explaining the scientific reasons for the disagreement, without going into the personal conflict between Warburg and Emerson, and the psychosis that drove Burk again.

Three points need to be taken into consideration. First, the importance of the subject. It was not simply an arbitrary number that was being questioned, but a key parameter on which the modelling of the photosynthesis mechanism would be based. Thus, finding the true value was of great significance. Second, the complexity of the debate. The experimental difficulties the researchers faced were enormous, in particular the impossibility of differentiating clearly between the gas exchanges caused by photosynthesis and the gas exchanges caused by respiration. As Rabinowitch wrote in 1945, the possibility of a photo effect on respiration was "a nightmare oppressing all who are concerned with the exact measurement of photosynthesis".[215] This was a serious problem, and although all the protagonists acknowledged its existence, they were unable to solve it. Third, the strong dependence of the efficiency of photosynthesis on a vast number of interrelated factors. It was only at very low light intensities that the system approached its maximum efficiency; but in addition to that, it was found that the algae were extremely adaptable, and reacted in totally unforeseeable ways to the slightest changes in parameters. This meant that all sorts of quantum yields could be accurately measured – a statement with which Emerson and Warburg would have happily concurred – although none of these values might be the maximum quantum yield or the minimum quantum requirement. On the other hand, one had to make sure that the highest yields that were measured (in this case by Warburg and Burk) were yields that reflected photosynthetic oxygen production rather than secondary processes or methodical artefacts. This all led to the fact that, as late as 1960, Bessel Kok stated, in a comprehensive paper on the problem: "Preponderate evidence seems to support the generalisation that at least eight quanta are required per one O_2 evolved [...]. It is rather dissatisfying that 25 years after Warburg and Negelein's first estimations we cannot justify more firmly stated conclusions."[216] Kok felt that claims of requirements lower than six could definitely be refuted; he was less positive, however, when it came to values that were lower than eight.

In principle, the situation was not that exceptional; it is not unusual for scientists, at any given time, to disagree with each other. However, in most cases the disagreement is either settled within a limited time span or it is put aside to be solved by future generations. This was how the formaldehyde question was treated: it was not resolved – neither positively nor negatively – but it was dropped for a while. The list of priorities was revised, in view of the range of means available, and this resulted in a downgrading of the goal "to establish the causal relevance of formaldehyde as an intermediate in photosynthesis". The goal of finding the maximum quantum yield of photosynthesis was originally a subordinate goal - just

[214] Cf. Walker (1997), p. 8.
[215] Rabinowitch (1945), p. 569.
[216] Kok (1960), p. 623.

one of the goals that photosynthesis researchers had to reach in order to attain the superordinate goal of finding the appropriate model. It had been regarded as an important parameter on which to base modelling decisions, and thus not a minor question. However, at least for Emerson and Warburg–Burk, the subordinate goal became a goal in itself, so that to a certain extent they lost all sense of proportion.

It is rather telling that, starting from perhaps around 1950, and definitely after 1955, the photosynthesis collective lost its patience with the debate. The 1953 photosynthesis review written by Brown and Frenkel, in which the authors pleaded that the controversy be resolved using other methods, has already been mentioned.[217] Attention has also been drawn to the fact that the second Gatlinburg Conference on photosynthesis of 1955, which was explicitly devoted to the discussion of the most pressing issues in photosynthesis research, deliberately excluded any discussion of the quantum yield. It was also in 1955 that Warburg requested, for the third time, that the question be settled by an officially implemented, "impartial" committee. (The first and only time that impartial observers were actually used was when Burk and Hearon acted as independent observers at Urbana; Warburg made another, unsuccessful, request for an impartial committee to be set up before the start of the first Gatlinburg conference in 1952, which Burk felt was unnecessary.) It is difficult to interpret Warburg's motivation behind this idea. The easiest, albeit non-charitable, answer is that one person (or a small group of people) can be more conveniently worked on than a large and uncontrollable collective. The chances of success for a person such as Warburg, willing to apply appropriate techniques of persuasion, was comparatively high – and even if the impartial observers were not fully convinced (it is, for example, not known what Hearon's thoughts on the Urbana meeting were), it would be simple for Warburg to take advantage of any uncertainties. However, more charitable alternatives are conceivable, although it is hard to imagine that Warburg was naïve enough to think that a solution could be advanced by this very Prussian way of arriving at a top-down truth sentence.

The response of the collective, which was represented by the members of the NAS of the United States to which Warburg's request for an impartial committee was forwarded, was unambiguous. Scientific controversies had to be resolved by the collective, and short cuts were not permitted. It was bad enough that, throughout the controversy, Warburg had continuously neglected other scientific conventions. But Warburg's appeal that a superordinate committee take charge was on no account acceptable. From a meta-analytical perspective, the collective was well able to deal with the challenge. The matter was taken seriously for a long period of time, and the reactions came from a number of different quarters, each individual reacting according to his or her competencies. Thus, Franck dealt with the question from the point of view of theoretical physics and photochemistry, Emerson tried to sort out the problems that arose from the technique of manometry, while Arnold and others developed alternative methods to revisit the question independently. The huge amount of correspondence that was generated and the highly increased frequency of meetings aptly reflects the participants' wish to solve the problem cooperatively (even though the outcome was far from clear: contrary to what

[217] See Brown & Frenkel (1953).

Warburg and Burk believed, Emerson's friends and colleagues did not only support his point of view.)

An important way of resolving controversial questions such as the quantum yield problem was for researchers to meet and carry out experiments together. This common practice was repeatedly alluded to in this chapter, and will also feature in the remaining chapters of this book. Although the exchange of papers and letters with more detailed inquiries and arguments was central to the collective's collaborative work, it was not always sufficient. Many participants believed that a meeting in person was indispensable, particularly in those cases in which the subtleties of the method were controversial. It was less a matter of "tacit skills", rather that one can only report in letters and publications those details that one had regarded as being potentially relevant. Kok and others visited Burk's laboratory, on the one hand as a way of checking the results, that is, they wanted to see whether the published results really had been obtained, and on the other hand to witness Burk's working methods for themselves and to ascertain whether he had perhaps failed to mention (or even to notice) a detail or so of his experimental practice that could have had a decisive influence on the outcome, and would have explained the miraculous measurements.

Even though Warburg accepted Emerson's invitation to Urbana, he did not comply with the conventions of this type of encounter. Part of Emerson's disappointment and exasperation was that, once Warburg had arrived, the latter refused even to think about experimenting together on the quantum yield question. Indeed, one could conclude from this that Warburg had never intended to collaborate with Emerson to this effect, but simply had been looking for a temporary location at which to carry out his experiments (since he no longer had a laboratory in Germany).[218] It was only during the very last month of Warburg's stay at Urbana that Emerson finally succeeded in persuading Warburg to work alongside him and look together at the same set-up, the same algae, the same manometer readings. The collective tolerated Warburg's behaviour for a surprisingly long time – perhaps they felt that, notwithstanding Warburg's arrogance and personal flaws, they could not safely ignore him or his opinion on the controversial issue. Gaffron, for example, was still trying to keep Warburg in the USA in 1949, although he knew very well about the latter's difficult character and intolerable conduct towards Emerson at Urbana. The gain in scientific terms seemed to have outweighed these drawbacks (and, of course, Gaffron was indebted to Warburg for the help that the latter had given him in finding a new place to work in the 1930s). However, by the mid-1950s the situation had changed. Warburg and his co-workers had consistently failed to demonstrate the actual relevance of the factors that they declared were essential for observing high quantum yields, while his opponents had succeeded in demonstrating why certain factors would produce high yields as an artefact. Thus, the collective lost interest in Warburg's contributions. In addition to this well-founded lack of interest, those people who were directly involved in the debate had started to distrust both Warburg and Burk. At the beginning of 1955 Gaffron

[218] Cf., e.g., the letter Warburg wrote to his sister Lotte in 1946, in which Warburg mentioned that he might consider going abroad as a guest to some laboratory or other. See the quote on p. 187.

wrote to Franck that he had received a letter from Emerson stating that although Emerson had again faithfully reproduced the latest experimental set-up specified by Warburg, he had obtained no change in resulting quantum yields. Gaffron commented:

> He [Emerson] can't understand Warburg's results – and neither can I, of course. Unless one leaves the realm of science and says: since he [Warburg] lies anyway – he lies consciously when citing other people – why not also here? If his set-up has been worked on for so long, that it yields inaccurate results automatically, who will be able to detect this, without taking everything apart and building it up again, piece by piece. [Carl] Neuberg, who always lied, and may therefore be considered an expert in this matter, said, if one fails to prove that he [Warburg] has been deceiving us, then he will have won.[219]

Emerson had likewise come to believe that not everybody was playing by the rules. When Warburg claimed, in 1954, to have found low quantum yields in a carbonate buffer solution, Emerson was persuaded that there was a flaw in his methods, although it had been carefully concealed; and he suspected the same of any other surprising data that originated from Warburg's laboratory. On 20 May, Emerson wrote to Daniels:

> I feel as you do that maybe someone ought to check on Warburg's electrometric results, but I think there is a limit to what we can accomplish by checking each fantastic claim as it comes along. I'm afraid I have built up a prejudice against Warburg's experimental work, because of his abuse of the manometric technique, and I tend to feel that if I took the time I would find a joker in his electrometric measurements as well. One cannot print this sort of thing, of course, nor say it for the record. However, for the present I think I shall let the electrometric results alone, and see what I can find through further study of the transients as revealed by manometric measurements.[220]

These sentiments were shared by a number of other people, and his reputation suffered accordingly. For example, Warburg's finding that very small amounts of carbon dioxide were necessary for photosynthesis to function was unjustifiably swept aside.[221] It was only when Warburg raised the issue of the curious effects of blue light that Emerson became interested in the former's work again, but this was because the publication reminded Emerson how, many years earlier, Lewis and he had also found that the light in this region had strange effects, for example on the course of respiration, although they had never cared to scrutinise it in detail.

[219] Gaffron to Franck, dated 2 Feb. Franck, James. Papers, [Box 3, Folder 7], Special Collections Research Center, University of Chicago Library. The year 1955 was reconstructed from the description of Warburg's setup, namely catalytic use of blue or green light, which Warburg had first announced in Warburg et al. (1954).

[220] Emerson to Daniels, 20 May 1954, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Daniels, Farrington, University of Illinois Archives.

[221] See Stemler (2002) for a short review of the so-called bicarbonate effect, discovered originally in Warburg & Krippahl (1958), and the detrimental effect Warburg's reputation had on the reception of this phenomenon. The bicarbonate effect became an important theme of research; it was predominantly pursued in Govindjee's laboratory at Urbana, and was the theme of seven PhD theses that he supervised. See, e.g., van Rensen, Xu & Govindjee (1999) for a survey of this work.

Warburg's finding thus prompted Emerson to return to this phenomenon, for which he used Warburg's two-light beam approach. And luckily so, one might add, for otherwise Emerson would not have hit upon the Enhancement Effect so quickly and any delay would have meant that someone other than Emerson would have made the discovery.

Finally, I would like to draw attention to the fact that, although few people were able to replicate Warburg's results, this was not generally regarded as a sufficient argument in the debate. Although it was generally felt that the more people who failed to get the high Warburgian quantum yields, the less probable it was that everybody was wrong and that only Warburg was right, this situation was at best a stalemate, and it certainly did not clear up the controversy. Recall the statement that Brown made to Emerson: concerning Emerson's argument with Burk, Brown reminded his colleague that it was not enough to state that "we haven't obtained these results", because the answer would always be, "then you had the wrong conditions" (see the quote p. 225). At best negative data should be understood to indicate that something is suspect. What was needed was the approach that Emerson took, first with Lewis, then later with Chalmers: find out how Warburg's data could be replicated and then *explain* why in order to obtain these results, one had to use methods that were flawed in certain respects.

What role did this controversy play in the history of modelling photosynthesis? It was surely an issue that consumed a great many hours of experimentation and thought, and it is often conceived of as a great stumbling block, which effectively prevented the field from progressing much faster. Although there is some truth in this statement, I feel that it is not entirely justified. One should not forget that, while this controversy was running its course, photosynthesis research was being strongly promoted in the laboratories of, for example, Hill, Calvin, Benson and Arnon. It was also pointed out in this chapter that, thanks to the debate, many insufficiently appreciated aspects of photosynthesis came to the fore. The body of knowledge of the process as a whole was thereby greatly advanced, even if this knowledge was not immediately integrated into any new comprehensive model suggestions. The controversy attracted scientists from widely divergent methodical competencies: Brown, Brackett and Daniels, who, up to then, had previously not worked in photosynthesis, ended up making valuable contributions to the field and inspiring others to continue with their photosynthesis studies. (It is possible that Brown and Brackett would have contributed to the field without the pending question of the maximum quantum yield; yet these counterfactual scenarios do not help us to evaluate the situation.) On the other hand, of course, others were discouraged by the course that the debate took. Max Delbrück, for example, had at one point seriously considered taking up photosynthesis research instead of bacteriophage studies, but decided against it, since he was thoroughly put off by the poisoned atmosphere of the conferences.[222] However, considering the seminal contributions Delbrück made to molecular genetics, this decision should be regarded as a particularly fortunate one.

[222] Cf. *Max Delbrück: Oral History Interview with Carolyn Harding, July 14-September 11, 1978, CIT, p. 55, <http://resolver.caltech.edu/CaltechOH:OH_ Delbruck_ M> (n.d.)*, p. 105.

I would like to close the chapter by citing a penetrative limerick about Warburg, written by an anonymous contemporary, which requires no additional comment:

> There was a great scientist named Otto
> who lived by the following motto:
> "I am always right!
> My enemies I'll fight.
> (But I'll be glad to send them my photo.)"[223]

[223] Quoted in Höxtermann & Sucker (1989), p. 106.

Chapter V

ELUCIDATING THE DARK REACTIONS (1937-54)

While the quantum yield controversy was unsettling one half of the photosynthesis collective, the other half was, at the same time, deeply involved in studying the so-called "dark reactions" of photosynthesis. Although the two sides of the collective obviously overlapped, they also differed to a surprising degree. In the late 1930s, a rather neat process of labour division had separated the photosynthesis collective into those who were concerned with the photochemical stage of photosynthesis and those who dealt with its thermochemical reaction, which, by that time, was generally agreed to be the process of carbon reduction. The turning point came with the advent of radioactive isotopes (or radioisotopes), as it was found that they could be used to trace the metabolic processes of plants and animals. In particular, it was the discovery of the long-lived isotope carbon-14, made by the United-States-based Martin Kamen and Sam Ruben in 1940, that lay the groundwork for the subsequent elucidation of many metabolic pathways, including that of photosynthetic carbon reduction.

This chapter starts with a discussion of the path-breaking work done by Kamen and Ruben, and then continues with an analysis of the research team headed by Melvin Calvin at the University of California, Berkeley (US); it was Calvin, who, together with Andrew A. Benson, succeeded in elucidating the reaction cycle. Through sheer manpower and financial support (generously provided by the United States Atomic Energy Commission [AEC]), this group rapidly outmatched all its competitors, including the University of Chicago team led by Hans Gaffron. However, it was not an easy game to play: competition and controversy reached, at times, atrociously high levels. Calvin would suffer two heart attacks before the Berkeley project was completed, and one of Gaffron's close collaborators, Edward Fager, left photosynthesis research for the more amicable atmosphere to be found in animal ecology.[1] Kamen remembered that during this period he tried hard to avoid attending any meetings at which photosynthesis might be discussed, simply because he would, much to his dismay, be inevitably called in to mediate should contradictory results lead to conflict between the parties.[2]

The second half of the chapter reconstructs the work of the Berkeley group: first, I shall explain how this collective, whose working methods were, at the time, rather unusual, operated; then, I shall outline the various proposals that the team made to the photosynthesis model, which were published by Calvin's laboratory in a series of no less than twenty-one publications, all of which were entitled "The Path of Carbon in Photosynthesis". The major part of the group's work on the reaction cycle was completed by 1954, for which Calvin was awarded the 1961 Nobel Prize in Chemistry, although no acknowledgement was given to the equally central

[1] See Kamen (1985), p. 193.
[2] See Kamen (1985), p. 205.

contributions made by Calvin's collaborator Benson and Kamen, the discoverer, with Ruben (who had by then died in a laboratory accident) of carbon-14.[3]

1 Martin Kamen, Sam Ruben and the Rat House (1937-44)

1.1 Kamen meets the Cyclotron

It may come at a surprise that it was the cyclotron that actually paved the path for the elucidation of the fate of carbon during photosynthesis, and, in particular, the first cyclotron in the laboratory of Ernest O. Lawrence. It has been told many times before how Lawrence, at the age of twenty-seven, arrived at the University of California's Berkeley campus in 1928, and how he set up a unique, interdisciplinary laboratory that became well-known for being the first example of so-called "Big Science".[4] The instrument in question was a type of particle accelerator, which was referred to by Lawrence as the "proton merry-go-round" but which publicly became known as the "cyclotron". However home-made and clumsy the first versions of this instrument appeared (the prototype was constructed of glass, sealing wax and bronze), they still operated perfectly well. The cyclotron worked as follows: charged particles were spun around in a vacuum chamber by means of a high-frequency, alternating voltage combined with a steady magnetic field. This resulted in an enormous increase in the energy of the particles, which made them go round in spirals, until, finally, they were thrown upon a target at the perimeter of the chamber. These deliberately induced collisions created secondary particles, which could then be extracted for analysis.

The first of these instruments contained an accelerating chamber that was no more than five inches (12.7 cm) in diameter; it was followed by a series of ever growing variants, which finally exceeded the capacity of Lawrence's laboratory. Lawrence managed to persuade university officials that he needed more space, and in 1931 an empty building with a sufficiently sturdy floor was turned over to Lawrence into which the new 27-inch (68.58 cm) cyclotron (which included a seventy-ton magnet) was moved: this became the famous Berkeley Radiation Laboratory. From the outside, no one would have suspected that the clapboard building housed a laboratory with cutting-edge technology. However, compared with the fancier-looking new buildings of the university, it had the enormous advantage of having a solid concrete floor which was strong enough to support the enormous weight of the new cyclotron. It was in this building, affectionately called the (Old) Rad Lab, or ORL, that the complete route that carbon travels through a plant during photosynthesis was later mapped.

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[3] The story of how the path of carbon in photosynthesis was elucidated has been told many times before. See, e.g., Bassham (2003), Benson (2002*b*), Benson (2002*a*), Calvin (1992), Calvin (1964), Calvin (1989), Florkin (1979) (Chapter 56, pp. 81–108), Kamen (1974), Kamen (1985), Kamen (1989), Lehmann (1968) and Morton (2007). Rabinowitch (1956) also contains a section on the "Evolution of the CO_2 Reduction Mechanism", in which the different stages of the work carried out at Berkeley is summarised (pp. 1688–1698).

[4] On Lawrence, the Berkeley Radiation Laboratory and the early history of the cyclotron, see, e.g., Heilbron & Seidel (1989), Heilbron, Seidel & Wheaton (1981) and Herken (2002). For a more general view that enlarges, e.g., on the links with the Manhattan Project, see also Boorse, Motz & Weaver (1989) and Rhodes (1986).

Figure V.1: The original clapboard Radiation Laboratory (Rad Lab), which later became known as the Old Rad Lab or the ORL for short, of the University of California, Berkeley.

In 1935, Lawrence invited his brother John, a physician, to start a biomedical research unit at Berkeley in order to explore how the radioactive isotopes, which were being produced in large quantities by the cyclotron, could be used. Early on in these studies, radioactive phosphorus-32 was unexpectedly found to be able to cure mice suffering from leukaemia, and in 1937 John Lawrence succeeded in improving the condition of a human (who was suffering from a rare bone marrow disease) using the same means. This newly discovered therapeutic use gave Ernest Lawrence more than enough justification to raise money for an even bigger instrument: the 60-inch (1.52 m) cyclotron, with a 200-ton magnet, for which, again, a new building was required – the Crocker Laboratory – and which saw its first run in 1939. In that very year, Ernest Lawrence was awarded the Nobel Prize in Physics, as stated by the Nobel Prize committee, "for the invention and development of the cyclotron and for results obtained with it, especially with regard to artificial radioactive elements".[5]

During the second half of the 1930s, which was a particularly turbulent period from the point of view of nuclear physics, photosynthesis researchers also began to use the cyclotron. It all began with a young chemist named Martin D. Kamen, who started working in Ernest Lawrence's laboratory in 1936 (see fig. V.2). Born in Toronto, Canada, but brought up in Chicago, Kamen was the son of Russian

[5]The laudation can be retrieved from the official website of the Nobel Prize committee at <www.nobelprize.org>.

immigrants.[6] He received his PhD in Physical Chemistry from the University of Chicago in 1936, after which he went to California to ask Lawrence for a job. Kamen seems to have been the right person in the right place at the right time. After he had worked in the laboratory for six months, with enthusiasm and skill but without a salary, Lawrence realised that a chemist was, after all, clearly needed to oversee the preparation of the radioisotopes. In the first place, John Lawrence's biomedical group had to be supplied with radiophosphorus; but the laboratory was also receiving an increasing number of requests for radioisotopes from outside the university. In view of these developments, Kamen became the only person in the laboratory to be employed on a permanent contract; and although his working hours were largely filled with time-consuming technical tasks, such as maintaining the cyclotron and distributing the isotopes, Kamen still managed to find time to carry out his own research.

In his autobiography, Kamen gives a lively description of the early years of the Rad Lab. In line with other accounts of the congenial working atmosphere in Ernest Lawrence's group, Kamen repeatedly emphasises the highly collaborative, pioneering spirit that prevailed in the laboratory: "It is impossible to describe the enthusiasm and zeal for accomplishment that pervaded the Radiation Laboratory in those magical years," Kamen writes, continuing: "A common determination to make the cyclotron bigger and better actuated all of us, producing the warmest camaraderie."[7] Kamen believed that the laboratory provided the ideal environment for creative work, "even though the emphasis on keeping the machine running seemed exaggerated at times".[8] In fact, the practical problems of running the first cyclotrons, and the strain put on the group by Lawrence's insistence that a new and bigger instrument be constructed every couple of years, were enormous. Kamen felt that the high proportion of technical work was, perhaps, one of the reasons why a number of major discoveries were not made in the Rad Lab, with the cyclotron directly at hand, but elsewhere: induced artificial radioactivity and the neutron, the phenomenon of nuclear fission, and the identification of several hitherto unknown isotopes and elements were all discovered in other laboratories. However, a sufficient number of discoveries were still made at Berkeley, to which Kamen made decisive contributions.

1.2 Kamen meets Ruben

In 1937, when he had still only been at Berkeley for a few months, Kamen bumped into another young chemist, Samuel Ruben (see fig. V.3, p. 256), who was finishing his PhD work in Berkeley's Department of Chemistry and had his desk in the so-called "Rat House". The latter, located in the neighbourhood of the Rad Lab, was officially known as the Chemistry Annex; it was built in 1915 and had been little refurbished since then.[9] The two young men, both at the time twenty-four years of age, decided to collaborate on the chemically interesting aspects of the cyclotron's

[6] See Kamen (1985) for his full autobiography and Kamen (1989) for a shorter perspective. See also Gest (2005) for a personal tribute to Kamen by Howard Gest, who was Kamen's first PhD student at Washington University, St. Louis.

[7] Kamen (1985), p. 70.

[8] Kamen (1985), p. 73.

[9] On the life and work of Ruben, see, e.g., Gest (2004) and Johnston (2003), Chapter 3.

Figure V.2: Martin Kamen (1913–2002), c. 1939. Original preserved in the E. O. Lawrence Berkeley National Laboratory (Seaborg Archive).

output, which they believed were being unjustly neglected by Lawrence's physics-dominated group. Their collaborative work very soon took a decisive turn. Kamen described in his autobiography how one day Lawrence dashed into the laboratory, in a highly excited state of mind, bringing him the young Assistant Professor in Physiology, Israel L. Chaikoff:

> E.O.L. [= Ernest O. Lawrence] informed me that Chaikoff had a proposal to use short-lived carbon-11 to study carbohydrate metabolism. [...] When I inquired how this was to be done, particularly how the ^{11}C could be incorporated quickly into the starting material, such as D-glucose, E.O.L. said the isotope would be given to green plants as CO_2. By photosynthesis the plants would in short order synthesize the radioactive glucose, which could then be fed to the rats used in Chaikoff's studies.[10]

Although Kamen immediately agreed to join the project, and enthusiastically promised to provide as much carbon-11 as was required, he was soon to learn that it had in fact been his friend Ruben who had originally come up with this suggestion: "He had mentioned it to Chaikoff and was incensed that the latter seemed to be passing it off as his own [idea]."[11] However, the matter was promptly cleared up, and work on the project started. Yet, what from today's vantage point might

[10] Kamen (1985), p. 81–82.
[11] Kamen (1985), p. 82.

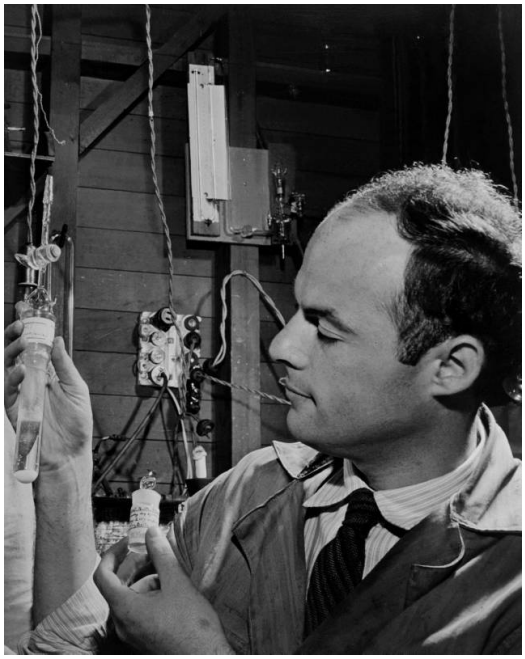

Figure V.3: Samuel Ruben (1913–43) in the Rat House. Original preserved in the E. O. Lawrence Berkeley National Laboratory (Seaborg Archive).

appear as a self-evident method of working – two chemists working cooperatively with a biologist – was highly exceptional at the time: the Berkeley chemists usually considered biology to be a second-rate discipline. Kamen was in a relatively safe position in the Rad Lab, but Ruben was only a young lecturer, employed on a limited, fixed-term contract by Berkeley's extremely competitive Department of Chemistry. Considering his situation, it was rather bold of Ruben (some would have said foolish) to pursue a line of research that could potentially harm his reputation as a chemist, and thus have an adverse effect on his academic career.[12] Nevertheless, Ruben chose to embark on this project with Kamen and Chaikoff – without knowing, of course, that his decision would prove to be of major importance to the future of chemistry on the Berkeley campus.

Ruben's confidence that the risk would pay off was not without foundation. Elucidating the pathways of intermediary metabolism had been the superordinate goal of biochemists and physiologists since the nineteenth century; yet, while some advances had been made by the early 1930s, it was still not understood how the vast majority of metabolic processes functioned. The insurmountable problem was the lack of direct access: it had proved impossible to study the processes in any "normal" chemical sense. Whenever the usual analytical techniques were applied,

[12] In his autobiography, Kamen stressed that Ruben's reputation was boosted when, after the latter had achieved some visibility in the department through the isotope studies, some graduate students chose to work with him on strictly physico-chemical problems, so that it became apparent to the department that Ruben was not exclusively committed to biochemical work. See Kamen (1985), p. 114.

the processes under study stopped operating. A crucial step forward was only made in the mid-1930s when (stable) isotopes were introduced to metabolic studies. In 1932, the chemist Harold Urey, working at Columbia University in New York, had discovered the heavy hydrogen isotope "deuterium" (^{2}H or D) and succeeded in the preparation of "heavy water", that is, water incorporating deuterium instead of the usual hydrogen.[13] In 1934, Rudolph L. Schoenheimer, a biochemist of Jewish ancestry who had been dismissed from his academic post at the University of Freiburg, Germany, by the Nazi Government, obtained a position at Columbia, and together with his young co-worker David Rittenberg, started investigating the biological implications of Urey's discovery; and after a short while Schoenheimer devised the isotopic tracer technique, which enabled researchers to study metabolic processes in detail. The trick was that compounds, into which heavy isotopes of either hydrogen (D) or nitrogen (^{15}N) were incorporated, were then rendered identifiable, for example, by spectroscopic methods. It was for this reason that the technique came to be called "tracer" methodology: it enabled scientists to *trace* the compounds that had incorporated the isotopes and, hence, the intermediate steps of biochemical pathways. Schoenheimer and his group were extremely successful, particularly after they had found out how label compounds twice (by the use of two different stable isotopes); this proved to be an ingenious method for investigating potential group transfers in a pathway. The findings were astonishing: they opened up a vast range of new experimental approaches and eventually led to a completely new concept of the cell's metabolism, which involved the idea that the cell's constituents were not static but in a dynamic state of continuous turnover.[14]

Ruben was certainly aware of these crucial developments, as was everybody else working in the field of isotope chemistry at the time (in the US at the very least). It took only a short leap from the successful use of *stable* isotopes to the idea that *radioactive* isotopes could also be useful in metabolic studies. Making use of the recently found radioactive carbon isotope carbon-11 seemed particularly promising, not only in view of the central role of carbon in metabolism but also because one could apparently easily produce "tagged" glucose in photosynthesis: if radioactive carbon dioxide were fed to green plants in the light, this radioactive compound should (hopefully) incorporate the radioactive label in all six carbons of the glucose; and these in turn should then remain identifiable through all the other intermediate stages of the metabolic process. The dream of biochemists from the time of Liebig seemed to have become a reality! Thus, it is not particularly surprising that Ruben was highly intrigued by the idea of using consistently labelled glucose to determine conclusively, for the first time, how animals use sugar in metabolism.[15]

The only obvious drawback of this approach was the short half-life of the available isotope carbon-11 (which was just under twenty-one minutes), but some

[13] Urey was awarded the 1934 Nobel Prize in Chemistry for these notable achievements.

[14] These studies' results were later summarised in Schoenheimer (1940). See also Simoni, Hill & Vaughan (2002) for a review of the work by Schoenheimer and Rittenberg; the classic papers are Schoenheimer & Rittenberg (1935) and Schoenheimer & Rittenberg (1937). Kohler (1977) provides a thorough historical study of the episode.

[15] See Kamen (1985), p. 82.

objectives still seemed possible.[16] From Ruben's point of view, and this is crucial, the superordinate goal – to gain a fundamental insight into the complex fate of glucose in the rat's digestive system – was within reach, if only Kamen (as the radioisotope expert) and perhaps somebody like Chaikoff (as the biologist) worked together, and if Ernest Lawrence (as the master of the cyclotron) could be persuaded to become interested in this plan. They were, after all, located in one of the best places in the world to carry out this kind of research, with a ready source of radioactive isotopes at hand; indeed, they were based in one of the few places in the world where a project of this type was feasible – and Ruben and Kamen were now sufficiently trained to be able to handle the isotopes. Their lack of expertise in animal and plant physiology was to be compensated for by the addition of two new members to the team: Chaikoff had already been recruited to the project and would bring with him his expertise in animal physiology; and plant physiology was soon to be covered by Zev Hassid, a friend of Kamen's who was working in the Life Sciences Division at Berkeley and who would be persuaded to join the project.

One can see the principle of research opportunism at work again here: if it seems that by investing a limited amount of time, resources and energy one can make a rewarding contribution to a field of study, why not go for it? It might not be your field of expertise, it might previously not have been on your agenda and you might not even have heard about the problem in general until very recently; but it might just be that the time is right for you to contribute your methodical knowledge or other skills (or temporarily join a group that is working on the subject) to a particular theme. The fact that this strategy pays off so well explains why scientists usually do not change their techniques and methods once they have reached a certain (usually advanced) stage in their career. The investment needed to acquire a comparable proficiency in a new method – say spectroscopy instead of manometry, or *vice versa* – would be disproportionately high. Instead, scientists are continuously on the look out for problems that they might be able to solve using the methods and techniques with which they are familiar. This is not only how Ruben and Kamen came to photosynthesis research, but also how James Franck stumbled into the field. However, while Franck would concern himself with questions of photosynthesis for the rest of his life, Kamen was brought, by the same line of reasoning (although under completely different circumstances), to the study of bacterial metabolism – a topic to which Kamen had never dreamed that he would contribute, but one that would, however, greatly benefit from the combination of his specific skills and interests. A prominent and well-researched example outside the field of photosynthesis research is the case of Hans Krebs and his discovery of the urea cycle: Krebs decided to examine how urea is synthesised in mammals because he considered that the question was well defined and not too complex, and, furthermore, one that seemed to promise almost immediate success, if examined using manometric methods. In the end, the solution turned out to be

[16] However, in Kamen (1949), one definite advantage of this short lifetime is pointed out, namely, that radioactive contamination was not a problem as all activity was completed so quickly. Half-jokingly Kamen also mentioned that "the investigator could not succumb to the temptation either to put off work or to start experiments without adequate protocols". (p. 367)

far more complicated than he had expected, but his principal strategy undoubtedly paid off.[17]

1.3 The First Metabolism Experiments

Ruben, Kamen, Chaikoff and Hassid immediately set to work, conducting the experiments as envisaged.[18] Boron-10 was chosen as the appropriate target material in the cyclotron, from which, after heavy bombardment, the radioactive carbon-11 was obtained and immediately combusted to $^{11}CO_2$. This whole procedure was taken care of by Kamen and Ruben, who also caught the gas in a U-tube immersed in liquid air. They would then dash off with the radioactive material to Ruben's laboratory, which was in the so called "Rat House" of the Department of Chemistry, where Hassid was waiting, so that he could administer the gas to his plants. About ten minutes after the $^{11}CO_2$ had been prepared, the leaves were cut off, chopped into pieces and immersed in boiling ethanol, in order to stop any further metabolic reactions from occurring. The radioactively labelled glucose (or related carbohydrates) was extracted from this liquid, and fed to Chaikoff's rats. The first problem was that only tiny amounts of radioactively labelled product were produced – far too few to be isolated using the standard methods in chemistry. Thus, the group had to make use of "carrier" molecules; in this particular case, they added ordinary, unlabelled glucose, so that a reasonable amount of precipitate could be obtained. The need to add these carrier molecules brought the group, of course, to the second obvious problem of the approach: the scientists had to predict in which compounds the labelled carbon would appear, otherwise they would not be able to detect the traces of labelled substance from the large amount of unlabelled material. To their utter surprise, Ruben, Kamen and Chaikoff learned that the series of intermediate steps in photosynthesis from carbon dioxide to glucose was far from well-understood.

It proved extremely hard to transform this procedure into a stable protocol. Not only were the yields of $^{11}CO_2$ unforeseeable; the next steps turned out to be even more erratic in outcome. Sometimes the plants absorbed sufficient amounts of the labelled carbon dioxide, sometimes not; and worst of all was that hardly any of the labelled carbon showed up in the glucose.[19] "In the meanwhile, Chaikoff and his expectant students were becoming restive," Kamen recalled.[20] Then, there was an unexpected turn of events:

> During a recital of these troubles Sam [Ruben] suddenly stopped, his eyes widened, and he blurted, "Why are we bothering with the rats at all? Hell,

[17] On Krebs's discovery (which he made with Kurt Henseleit), of the urea cycle, see the pertinent chapter in the seminal biography by Holmes (1991), as well as the alternative reconstruction proposed in Graßhoff et al. (2000), Graßhoff & May (2003) and Nickelsen & Graßhoff (2008).

[18] See Kamen (1985), p. 83, for a description of the early stages of the project.

[19] In retrospect, one can understand the nature of these two stumbling blocks : (1) Calvin and his co-workers later demonstrated that plants prefer "normal" carbon, so that radioactively labelled carbon dioxide tends to be absorbed at lower rates; (2) in contrast to standard (even very recently published) textbooks, Walker (2007) has underlined the fact that "like sucrose, free glucose is not a major product of carbon assimilation by photosynthesising chloroplasts if, indeed, it is formed *in the light* at all". (p. 182).

[20] Kamen (1985), p. 84.

> with you and me together we could solve photosynthesis in no time!" From that moment we were out of everything but the photosynthesis business.[21]

This remarkable moment, which Kamen remembered so vividly (and I trust that he did, even if he might not have recalled Ruben's exact wording), is one of those rare instances in which a change in a research goal can be precisely nailed down. Before this moment, the whole business of having plants fix the labelled carbon dioxide and turn it into glucose was only the means to another end, namely, to find out what happened to glucose in rats. Facing the puzzling results, above all the fact that the labelled carbon disappeared somewhere in the plant, but not in the form of glucose, Ruben, Kamen and Hassid realised that the means could be turned to an end in itself. What up to then had been thought of as being only a preparatory procedure to provide a measuring device (*Herstellungsprozess*) became the actual focus of investigation (*Untersuchungsprozess*).[22] As a consequence, a new experimental protocol was developed:

> Our strategy for the solution of the Big Problem [that is, identifying the first product of CO_2 fixation in plants] was simple in prospect, if complex in implementation: feed the plants $^{11}CO_2$, wait for predetermined lengths of time, from a few minutes up to an hour, add carrier (measurable amounts of whatever compound we guessed might be labeled in the $^{11}CO_2$ exposure period), extract, isolate, and see if any or all of the radioactivity appeared in the carrier. If we had guessed right, most, if not all, of the radioactivity would be localized in the compound defined by the carrier.[23]

Simple in prospect, if complex in implementation: this formulation appropriately catches the group's struggle during the next weeks and months. Straightforward as the principal idea sounded on paper, in practice it proved extremely challenging. Getting access to cyclotron runs was the first difficulty: the cyclotron was only occasionally available for the preparation of carbon-11 or other isotopes of biological interest, and never before 9-11pm; furthermore, after having completed numerous runs during the course of the day, by evening the cyclotron often suffered from technical defects and had to be fixed by Kamen before any more runs were possible. The next problem was to find a procedure that would provide enough labelled $^{11}CO_2$, without exposing the person in charge to enormous amounts of radioactivity. Even after improvements had been made to the procedure, Kamen was still so heavily contaminated with radioactive material after having prepared the isotopes, that Ruben banned him from entering the laboratory in the Rat House while samples were being counted. Finally, the short half-life of carbon-11 was a serious problem. After the labelled material had been retrieved, there was barely enough time to carry out all the necessary operations (since everything had to be done within the period of about two-and-a-half hours maximum). Kamen gives an excellent description of the manic speed at which they needed to conduct the experiments in his autobiography:

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[21] Kamen (1985), p. 84.

[22] See Graßhoff et al. (2000) for these terms.

[23] Kamen (1985), p. 84. Additional background information on these early experiments can be found in Benson (1982) and Barker (1982).

> At the Rat House, Sam [Ruben] and Zev [Hassid] would be waiting for me like sprinters at the starting gate. Beakers would be filled with boiling water or other solvents and pipettes ready to suck up measured volumes of radioactive solutions onto absorbent blotters, which would be held by tongs over hot plates and dried. All the necessary reagents and apparatus would be in place. The [Geiger] counter would be ticking away establishing the background activity. Each experiment had to be planned ahead in every detail so that no time was lost in confusion or delay in deciding what procedure to follow. Anyone looking in on the Rat House when an experiment was in progress would have had the impression of three madmen hopping about in an insane asylum, what with the frenzied activity punctuated by loud classical music from the radio monitor [to register the running of spark testers in other laboratories, which would have confounded the measuring], and Sam's yells to get on with it and hand him samples while he sat at the counter table, feverishly taking background and sample counts. We had no idea of what had happened until hours later when, with all samples assayed, we sat in exhausted consultation, calculating and evaluating the results.[24]

In addition to the frenzy taking place in the laboratory, the researchers spent many hours in the library learning about the almost infinite number of substances that could be potential intermediates in photosynthesis, in which, accordingly, the labelled carbon might appear, and looking up the analytical procedures for isolating each of them. (Note that formaldehyde was still one of the substances that Ruben, Kamen and Hassid tried especially hard to find!) The ever higher demands of the project, on time and physical strength, which came on top of everybody's routine work, finally caused Hassid to withdraw from the project: he suffered from high blood pressure, and was strongly advised by his physician to change his way of life.[25] Kamen and Ruben had, by then, acquired enough knowledge to handle the algae and bacteria themselves (after a short initial period during which they had worked with barley, the group had abandoned using higher plants), mainly thanks to a crash course delivered to them by the chemist-turned-microbiologist Horace A. Barker from the Department of Plant Sciences.[26]

Although Ruben and Kamen became increasingly proficient in their search for the reduction products in photosynthesis, they were keenly aware of their limited understanding of the biology behind it, and tried to learn as much as they could about other aspects of the process. Kamen emphasised in his autobiography how eager Ruben and he were to communicate with other photosynthesis researchers in the country, in order to exchange (and critically discuss) the most recent findings. One of the centres of photosynthesis research at the time was only a few hours' drive away from Berkeley: the Biological Division of the Carnegie Institution at Stanford, where in 1937 Robert Emerson, Charlton M. Lewis and Charles Stacy French were working on different aspects of photosynthesis, while during these years visitors such as Hans Gaffron and James Franck, completed the range of discussion partners at this first-rate institution. Hardly less important was the work going on at the Hopkins Marine Station of Stanford University at Pacific Grove, where Cornelis B. van Niel and, at the time, William A. Arnold were based.

[24] Kamen (1985), p. 86.
[25] Kamen (1985), p. 87.
[26] Kamen (1989), p. 140; Barker (1982), p. 68.

Kamen and Ruben were warmly welcomed into this Californian discussion circle. Far from showing contempt for their lack of biological training and photosynthetic expertise, the chemists' ideas were met with keen interest. Kamen remembered that Emerson and Arnold "reacted so enthusiastically when they were made aware of our prospects, going so far as to declare that all work should be held in abeyance until we had the chance to fully exploit our labeling techniques".[27]

As has already been mentioned in other chapters of this book, these informal meetings – of as many experts as could be gathered in one place – were highly characteristic of photosynthesis research in these years. In addition to the meetings themselves, much of the correspondence of the time included reports, to keep those who had been unable to participate in person informed. It is clear that the scientists benefited enormously from these meetings and the opportunity to exchange ideas and data freely. One might have thought that they would have tried to keep their ideas to themselves until they had been published, yet this was undeniably not the case. It would have been foolish, anyway, to miss the chance of discussing preliminary results in advance, so that, perhaps, a better-founded publication could be written; it was equally rewarding, in the long run, to contribute to the interpretation of other people's data, since one could be sure of receiving a similar amount of advice on other occasions. Indeed, there were only few instances in the years covered by this study in which questions of priority or scientific misconduct would cloud the friendly atmosphere of such meetings. The fact that the free exchange of information and opinions between scientists was vital is most evident from the difficulties they had when they were forced to adapt to the security demands placed upon them during the Second World War.[28]

The next few years were particularly rewarding for Ruben and Kamen, who became the established leaders in radiotracer methodology and highly requested speakers on the conference circuit. There was only one hitch, Kamen recalled: "Regrettably, we had not reached our goal of establishing the identity of the CO_2 fixation product, despite the many hundreds of experiments we had performed."[29] (The combination of methods that later proved to be the key – notably combining the long-lived carbon-14 as a tracer with paper chromatography – had yet to be developed.) However, in spite of the severe methodical limitations, by the end of the 1930s they had made some substantial discoveries:

> Our experiments clearly established the existence of two systems, one a complex of dark reactions for CO_2 uptake with production of reduced cell material, and the other a light dependent process for the simultaneous evolution of molecular oxygen. These two systems in the plant had to be closely coupled so that one did not get ahead of the other, but the means for accomplishing this still remained unknown. [...]
>
> We [furthermore] showed in our tracer studies that [the first fixation product in photosynthesis] was a compound formed by a readily reversible reaction in the dark whereby the CO_2 could react with the primary acceptor or acceptors to form carboxyl [R-COOH]. Further, we showed that as the

[27] Kamen (1985), p. 104. It is possible that Kamen meant Lewis, who at the time was working with Emerson, and confused him with Emerson's earlier collaborator Arnold.

[28] Kamen (1985), p. 151.

[29] Kamen (1985), p. 106.

> time of exposure of such labeled products to light was increased, less activity appeared in the carboxyl and more in carbon reduced further in an irreversible manner.[30]

The importance of establishing that carbon dioxide was reduced in the dark should not be underestimated. It was still widely assumed that carbon dioxide reduction was achieved by the action of light on a complex with chlorophyll (see Chapter III). However, the data produced from the radioactively labelled carbon did not support this theory. Furthermore, as mentioned in the quote, Ruben and Kamen concluded from their data that, most probably, the primary fixation product was a charged molecule with carboxyl and hydroxyl groups, that is, it was likely to be an aldehyde. A year later Kamen and Ruben tried to find out this compound's molecular weight, which was no easy task, given that the usual techniques for doing so required about a million times more of the substance than Ruben and Kamen had at hand. The two feasible techniques they resorted to resulted in widely divergent figures: while one indicated a molecular weight of 100 to 400, the other yielded a figure between 500 and 1000, and the prospect of acquiring more precise figures through substantial improvement of the technique was highly unlikely. It is interesting to note how Ruben and Kamen (mistakenly) settled on the higher figure:

> We knew from reading the literature that the reactions of carbon dioxide to form a carboxyl product were not favored unless the molecules reacting were complex [i.e. large], such as in certain polyphenols. In these cases, the reactions to form carboxyls might occur significantly at high temperatures, such as 200°C. We assumed that in the algae special conditions existed that made possible such reactions at room temperature.[31]

Ruben and Kamen decided that the required size and complexity of the reactants (which the literature claimed was a precondition for the reaction to occur) meant that a molecular weight of between 500 and 1000 rather than between 100 and 400 was more likely. The assumption that "special conditions" in the living system would make up for other factors necessary in experimental set-ups *in vitro* exactly parallels the reasoning that had dominated nineteenth-century chemistry: then it had also been felt that formaldehyde was formed in plants from carbon dioxide and water by the same mechanism (or a very similar one) that was operating in the test tube, even if the latter required extreme conditions. The vast difference that exists between physicochemical and biochemical systems only became clear to Ruben, Kamen and other interested chemists when the process of phosphorylation as the main source of metabolically usable energy (materialised mostly in the form of ATP) became widely known through the seminal papers by

[30] Kamen (1985), p. 107. Ruben and Kamen's research findings were summarised by Kamen in 1949 in a contribution to the volume edited by Franck and Loomis; see Kamen (1949). For the original papers, see: Ruben, Hassid & Kamen (1939*a*), Ruben, Kamen, Hassid & DeVault (1939*b*), Ruben, Kamen & Hassid (1940*a*), Ruben, Kamen & Perry (1940*b*) and Ruben & Kamen (1940*a*). In the 1960s, Don C. DeVault, discovered, together with Britton Chance, electron tunnelling processes in cytochromes; see, e.g., DeVault, Parkes & Chance (1967), DeVault & Chance (1966) and Blankenship, Amesz, Holten & Jortner (1989).

[31] Kamen (1985), p. 109.

Fritz Lipmann and Herman Kalckar.[32] A first attempt to model photosynthesis on the basis of these findings was published by Ruben, as sole author, in 1943.[33] In this paper, Ruben aimed to explain not only photosynthesis, but also carbon dioxide fixation in general:

> A new formulation of the mechanism of photosynthesis is briefly presented which offers a plausible model for the fixation and reduction of carbon dioxide not only for green plant photosynthesis but also for carbon dioxide fixation and reduction by the many different chemosynthetic and heterotrophic organisms.[34]

From his work with Kamen and Hassid, Ruben accepted as established knowledge that the first step in the reduction of carbon dioxide was the carboxylation of an organic residue. In addition, he argued that this process was not favoured energetically unless it was coupled with reactions that prompted an appropriate energy release. Drawing on the work done by Lipmann and Kalckar, Ruben suggested that the organic residue might first be phosphorylated by an energy-rich phosphorous donor, which would explain the rise in its energy level; and that in the second step the phosphorylated organic residue might react with carbon dioxide, yielding a carbonic acid, an inorganic phosphate and the necessary activation energy. Ruben further speculated that the unknown organic residue might be an aldehyde, and the energy rich phosphorous donor adenosine triphosphate (ATP), and he formulated accordingly a possible reaction sequence. As Ruben wrote:

> Thus by a sequence of coupled equilibria such as suggested above, the dark fixation of carbon dioxide may be accomplished (also suggested by Lipmann) at the expense of the hydrolysis of an energy rich phosphorylated compound.[35]

Although the details of Ruben's suggestion did not stand the test of time, the proposal that a charged, phosphorylated intermediate might be the carbon dioxide acceptor closely resembles what is known today about this reaction sequence. However, even Kamen, Ruben's closest collaborator, very much doubted that this scheme was correct. At the time, Kamen was unable, to see how light energy could possibly be used to produce energy-rich phosphates, and many of his colleagues shared this opinion.[36]

It is worth dwelling shortly on the discovery that the fixation, and at the same time the reduction, of carbon dioxide was not confined to plants but was generally present in a great many heterotrophic tissues, in organisms ranging from bacteria to mammals. A first inkling of this fundamental insight was provided by the celebrated publications written by the microbiologists Harland G. Wood and Chester H. Werkman, who found, towards the end of the 1930s, that propionic bacteria were capable of carbon dioxide fixation through the reduction

[32] Lipmann (1941), Kalckar (1941).
[33] Ruben (1943).
[34] Ruben (1943), p. 281.
[35] Ruben (1943), p. 280.
[36] Kamen (1985), p. 162. However, Ruben's reaction sequence did at least make Kamen think intensely about the general relationship between phosphate metabolism and biochemical energy storage, which later became a central theme of Kamen's research work.

of oxaloacetic acid to succinic acid.[37] In subsequent years, the generality of this phenomenon became slowly understood, and the work that Kamen and Ruben undertook with radioactive tracer carbon considerably contributed to the acceptance of this surprising insight.[38] The implications for photosynthesis research were enormous. If the fixation of carbon dioxide by plants was not exclusively connected to the light reactions of photosynthesis, then the compounds that were found to be radioactively labelled, and, hence, were interpreted as being derived from the labelled carbon dioxide, were not necessarily produced in the course of photosynthetic reactions.[39] Furthermore, it followed that the reaction mechanism for carbon dioxide reduction might be the same in bacteria, animals and plants, which discredited even further the still popular idea of the chlorophyll–carbonic acid complex as the site and catalyst of carbon dioxide reduction. This idea – to model carbon dioxide reduction in the process of photosynthesis along the same lines as carbon dioxide reduction in heterotrophs – was to play a central role in the debate on the path of carbon in photosynthesis, as will become clear later in this chapter.

1.4 Carbon-14 and the End of a Collaboration

The story of how Ruben and Kamen found, in 1940, the long-lived isotope carbon-14, that so fundamentally changed not only the study of metabolism but also innumerable other areas of science, has been told many times, by Kamen himself and by others, so that a sketch of the central events should suffice.[40] In view of the difficulties of working with carbon-11, there was a huge incentive to find a radioactive isotope with a longer half-life. The existence of such a carbon isotope had long been predicted, and there were indications that one did in fact exist, although no one had been able to isolate it. Ernest Lawrence was adamant that this discovery would be made in his laboratory, and he granted Kamen all the support and cyclotron time the latter needed to find this substance. Kamen enthusiastically seized the opportunity, and, together with Ruben, succeeded in discovering carbon-14 on 27 February 1940.[41] It took them some time, however, before they became thoroughly convinced of the accuracy of their discovery. Both Ruben and Kamen were terribly worried that they had made a mistake, even after the news had broken (Ruben refused to appear on the occasion of the discovery's public announcement at Berkeley), but their anguish was unfounded. Ruben and Kamen really had hit the nail on the head, and with it a new era in photosynthesis research (as well as in tracer methodology in general) began. Carbon-14 turned out to have a half-life of 5,600 years – more than enough time to carry out any biochemical experiment![42]

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[37] The seminal papers based on the study of propionic bacteria were Wood & Werkman (1935), Wood & Werkman (1936) and Wood & Werkman (1938). See also Singleton (1997) and Krebs (1974) for historical accounts of this important discovery.

[38] Ruben & Kamen (1940*b*).

[39] Cf. Kamen (1985), pp. 110–112.

[40] Kamen (1985), Chapter 7, pp. 122–146, is just one example; other references are given herein.

[41] The discovery was published in Ruben & Kamen (1941).

[42] The disadvantage of long half-lifes is, of course, that the number of disintegrations per minute becomes very small – sometimes too small to be detected. Fortunately, though, carbon-14 turned out to be still within the biochemically useful range.

Unfortunately, Kamen and Ruben were able to produce only a very small amount of carbon-14, so that only one single project with this new carbon isotope as a tracer was completed before December 1941.[43] In the same year, Kamen and Ruben started a collaborative project with the Berkeley-based physical chemist Merle Randall and the latter's graduate student James Hyde. Randall and Hyde had constructed a large distillation column, which allowed them to isolate heavy water in which the oxygen isotope oxygen-18 had been incorporated.[44] Among other aspects, Ruben and Kamen contributed to the project through their acquired expertise in handling the algae, which were then grown in a medium with a relatively high concentration of heavy water. In these experiments, the group found a strong indication that the photosynthetic oxygen did originate from the water, and not from the bicarbonate in the solution (although the data, of course, did not provide any information on the mechanism of oxygen release from water). The crucial finding was that the isotopic composition of the oxygen produced during photosynthesis was similar to the one in water but unlike the isotopic composition of oxygen in carbon dioxide and atmospheric oxygen.[45]

The attack on Pearl Harbor in December 1941 terminated any further pursuit of these lines of research. A range of other topics appeared on the chemists' agenda: Kamen became involved with the uranium enrichment process of the Manhattan Project, the US atomic bomb programme, while Ruben worked on a meteorological war project studying the biological effects of poison gas. Unjustly, however, once the war was over it was not Ruben and Kamen who were able to resume their highly promising work with carbon-14. In 1943 Ruben had died in a laboratory accident while experimenting with the highly toxic phosgene gas, and Kamen had become a victim of the McCarthy "witch hunts". Because of his alleged involvement with Communists, Kamen was fired from Lawrence's laboratory in 1944, and even though, once the war was over, he continued to make outstanding contributions to science at Washington University in St. Louis, Illinois, it took him about a decade to establish his innocence in court.[46]

2 Melvin Calvin enters the Stage

Thus, rather than Kamen and Ruben, it was the chemist Melvin Calvin who, in 1946, was made head of the newly founded Bio-Organic Chemistry Group

[43] This was a study in the metabolism of propionic acid bacteria, published as Carson, Foster, Ruben & Barker (1941).

[44] Kamen (1985), p. 140.

[45] This conclusion was arrived at independently by another scientific team, working in Russia at roughly the same time, which clearly indicates that: (1) many people jumped at the opportunities provided by the new methods; and (2) the pertinent problem was considered pressing and relevant. See Ruben et al. (1941), Vinogradov & Teiss (1941) and Vinogradov & Teiss (1947); a confirmation of the work done by Ruben et al. was given by Dole & Jenks (1944).) Warburg, incidentally, never accepted this conclusion. Note that Ruben et al. (1941) did not refer to the important work carried out by Robin Hill at the University of Cambridge (UK), which was described in Chapter III, section III.9, p. 151; nor did Hill refer to Ruben et al. (1941), although they had arrived at similar conclusions at roughly the same time – albeit from very different directions and using different methods. Either they were unaware of the other's work or they had decided, for whatever reasons, that their studies were not directly related.

[46] For more background information, see Kamen (1985) and Kamen (1989).

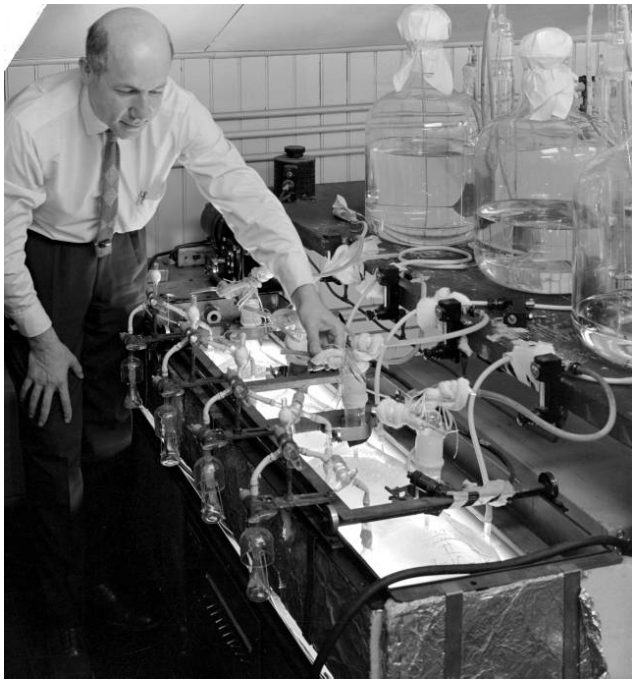

Figure V.4: Melvin Calvin (1911–97) with the Berkeley laboratory's algae cultures. (Note, however, that Calvin himself was never actually involved in culturing the algae).

at Berkeley. Although Calvin had been at Berkeley since 1937, he only became properly acquainted with Ernest Lawrence when the two of them were working together on the Manhattan Project. After the war was over, they met regularly for lunch at the so-called "physics" table at the Men's Faculty Club in Berkeley. Calvin himself circulated the story that, one day in November 1945, when the two men were walking back to their laboratories after lunch, Lawrence announced that "It's time to do something worthwhile!", meaning something beyond extracting uranium-plutonium fission products. The result of this announcement was the setting up of the Bio-Organic Chemistry Group, the task of which was to investigate the use of radioactive isotopes in chemical and biochemical studies. Lawrence suggested that this group study organic reaction mechanisms and the development of radioactive compounds to be used in the treatment of cancer, based on the carbon-14 produced by the cyclotron. Financial support would be secured from the AEC, with which Lawrence had excellent connections.[47]

In several respects, Calvin was an obvious choice for Lawrence, who needed to find someone to make the best of the Berkeley discovery of carbon-14. In 1935, Calvin had received his PhD in Chemistry at the University of Minnesota with a thesis on halogen electron affinity.[48] He had then gone, with a Rockefeller

[47] See Calvin (1992), p. 52; Seaborg & Benson (1997), p. 8.

[48] On Calvin's life and work, see, e.g., Loach (1997), Bassham (1997) and Seaborg & Benson (1997). See also Calvin (1989) and Calvin (1992) for autobiographical accounts.

Fellowship, for two years to the University of Manchester, England, where he spent most of his time in the laboratory of the renowned physical chemist Michael Polanyi. During these years, Calvin became interested in the electronic basis of the photochemical properties of porphyrins, such as haem and chlorophyll as well as their analogues. He went back to the US in 1937 to take up a lecturing post in chemistry at the University of California, Berkeley, where Calvin remained for the rest of his working life. In his first years at Berkeley, Calvin collaborated closely with the organic chemist Gilbert N. Lewis on the photochemistry of coloured porphyrin analogues and other themes. However, the war brought this work to a halt and Calvin, as mentioned earlier, was inducted into the Manhattan Project, where he became involved in the separation and purification of uranium and plutonium. By then Calvin had already acquired a reputation as an extremely intelligent and able scientist, with a broad range of interests and skills. One of his most remarkable traits was his ability (and audacity) to embark on totally new fields of science, armed with nothing but the natural ability to ask the right questions and quickly imbibe the answers. Consequently, Calvin was able to make contributions extremely quickly to the remotest theme on a surprisingly high level. He was an exceedingly fast thinker and would produce new models and theories at an almost terrifying pace; and he was not at all disturbed by the fact that, inevitably, a good many of his ideas turned out to be wrong.

In addition to his intellectual qualities, Calvin also proved to be a deft manager of his laboratory, and adept at selecting the right people for his group. In line with Lawrence's preferences, and with those of the AEC, the first projects of the newly founded Bio-Organic Chemistry Group focused on the medical applications of carbon-14, as well as on the synthesis of labelled amino acids and other metabolites, which were used in John Lawrence's laboratory. However, already from the start a subdivision was set up, headed by the chemist Andrew A. Benson, to specialise in photosynthesis research (see fig. V.5).[49] While the medically oriented part of Calvin's group settled in the newly erected Donner Laboratory, where John Lawrence's group was located, the photosynthesis division inherited the old clapboard building of Ernest Lawrence's original Rad Lab (which consequently was hitherto referred to as the *Old* Rad Lab, or simply the ORL). As mentioned earlier, the huge 60-inch (1.52 m) cyclotron no longer fitted into the wooden building, so that the cyclotron work had been moved to the Crocker Laboratory next door; soon thereafter, the smaller 37-inch (93.98 cm) machine was donated to the University of California, Los Angeles, Physics Department, so that the photosynthesis division could use the whole building. One of the major advantages of this arrangement was that, together with the building, the photosynthesis division also inherited access to the integrated glass shop, the carpenter's shop and the machine shop, including a number of skilled artisans who were eager to assist the scientists carry out their more extravagant ideas.[50]

[49] On Benson's life and work, see, e.g., Buchanan, Douce & Lichtenthaler (2007) and the autobiographical perspectives Benson (2002*a*) and Benson (2002*b*).

[50] It was James A. Bassham, in particular, who emphasised in retrospect the importance of collaborating with such highly skilled glassblowers, machinists and carpenters; see Bassham (2003), p. 38.

Figure V.5: Andrew A. Benson (b. 1917).

Inviting Benson, who was then at the California Institute of Technology (Caltech) in Pasadena, to become the head of the photosynthesis division was an obvious way to provide the necessary continuity to the studies undertaken by Ruben and Kamen before and during the war. As an conscientious objector (largely due to Emerson's influence), Benson had not been drafted, but had worked in 1942 and 1943 as a lecturer in the Department of Chemistry at Berkeley. In this capacity Benson had already collaborated with Ruben in the latter's carbon-14 studies (and also in the poison gas work). Thus, Benson was certainly qualified to continue with the research that had been so drastically terminated by the loss of both former experts, Ruben and Kamen. Benson's expertise was even more obviously needed when it became apparent that, first, Kamen had taken all his laboratory notes with him when he left and, second, that it was Benson who had received from Ruben a small vial containing carbon-14. It was only in the magnitude of millimicrocuries, as Benson recalled, but it was at the time the only supply of carbon-14 that existed.[51] (This situation, of course, changed rapidly after 1945, when radioactive isotopes became widely available in large amounts by virtue of the nuclear reactors.) Benson

[51] See, e.g., Benson (2002*a*), p. 34. Note that there is a discrepancy between Benson's and Calvin's accounts of who was given the vial: in Calvin (1992), p. 53, Calvin claims to have inherited this vial from Ruben himself. However, this version seems highly unlikely (why should either Kamen or Ruben have given the carbon-14 to Calvin rather than to Benson, who was a collaborator of theirs) and rather due to the fact that in his autobiography Calvin made no reference whatsoever to Benson.

became the leading scientist in the new photosynthesis laboratory, and it was mainly due to his skill and engagement that the infrastructure was set up in a comparatively short time and was of such extraordinary quality.

Benson designed the chemical hoods, laboratory benches and other facilities and instruments after the excellent laboratories at Caltech. He had the radioactively contaminated linoleum floor replaced, and made sure that all the benches were brightly illuminated. Benson also designed the legendary white table on which the chromatograms were later spread out for discussion and which very soon became the social centre of the laboratory. One of Benson's most ingenious inventions, which became particularly famous, was a special vessel in which the algae could most appropriately be illuminated and exposed to the radioactively labelled $^{14}CO_2$. Owing to its shape, this vessel became known as the "lollipop" (see fig. V.6). This is how Vivian Moses, one of Calvin's former collaborators, described this vessel in his recollections:

> A 'lollipop' was simply a glass vessel about 4 or 5 inches [10-13 cm] in diameter, circular in view, flattened so that the space between the two sides of the lollipop was relatively narrow – I would say something like 5 mm with an opening at the top for pouring liquid in and a large stopcock at the bottom. The idea was that you put the algal culture of *Chlorella* [...] in the lollipop, shone lights from both sides so that the algae were very highly illuminated, squirted in whatever radioactive material you wished to study the algal conversion of and, when you were ready to take a sample you opened the stopcock, which was a large stop-cock, and the liquid suddenly fell out straight into boiling alcohol and killed the plants very quickly, and, as it were, "froze" everything for later investigation. It was called a lollipop simply because it looked like the top end of a lollipop on a stick.[52]

Benson not only organised the technical side of the laboratory; he also coordinated the ongoing experimental work and was the creative source of many of the group's sub-projects. In a large-scale Oral History Project, carried out by the aforementioned Moses in collaboration with his wife Sheila Moses, a substantial number of scientists were asked about their recollections of working in Calvin's group. This is how in one of these interviews the chemist Murray Goodman, a graduate student at Berkeley from 1950 to 1953, recalled Benson's role in the laboratory:

> Absolutely and prime among them [i.e. among the senior scientists] was Andy Benson, who was very much a broadly based scientist in this area of photosynthesis and technically highly accomplished. He was in the laboratory at all times, so that he had that sense of how to make it happen in the lab. And, of course, discussions always involved him. He was, and is, a relaxed, contemplative person who is very easily accessible and in the day-to-day kind of world of being a graduate student or in functioning in [the] ORL, I would say that Andy had most of the responsibility for reacting to what was going on, analysing what was going on, suggesting routes to new experiments. That was a major emphasis of each day in the lab.[53]

[52] Moses & Moses (2000), interview with Moses, p. 17/6 [i.e. interview number 17 of the collection on page 6].

[53] Moses & Moses (2000), interview with Goodman, p. 14/11.

Figure V.6: Benson's famous "lollipop" vessel.

The third most important figure involved in reconstructing the path of carbon in photosynthesis was James Alan (Al) Bassham, who also joined the project at an early stage. Like Benson, he had been drawn to study photosynthesis through the influence of Ruben: in an interview session with Moses, Bassham related how, during his first year at Berkeley, Ruben had taken his turn to teach the practical component of a laboratory course to the chemistry students. One day, instead of discussing the protocols for the experiments they were to conduct, Ruben talked to the students about his research: how he and Kamen had recently acquired access to radioactive carbon-14 and now wanted to use it in their tracer studies. Ruben also told the students that Kamen and he wanted to trace the path of carbon in photosynthesis. Then the US joined the Second World War and Bassham enlisted in the US Navy for three years. On his return, he immediately enrolled at Berkeley again to undertake graduate studies. In view of his status as a veteran and his excellent academic reports, Bassham was taken on as a doctoral student straight away and given a list of potential supervisors. Bassham recalled the following:

> I got a list of professors to go to and Calvin was [...] the first one on the list. So, he was the first one I went to see and he provided me with a list of his research projects. The first one he mentioned was the work with carbon-14, both with photosynthesis and also with some organic reaction mechanisms. And, of course, being with the big professor I listened politely to all of his research proposals. But I had already made up my mind as soon as I heard about carbon-14 in photosynthesis, because of my experience with Ruben.

> And so that's the one I told him I wanted to work on and that's how I got started in that project.[54]

Before this encounter, Bassham had neither read anything by Calvin, nor had he even heard about him; Calvin was still far from being the major figure that he would later become. (It is clear from the interviews carried out by Moses that only a few of the people who came to work with Calvin were aware of his status in science. The younger scientists mostly came across his laboratory accidentally.) Noteworthy is the fact that Bassham was not assigned a theme for his thesis on which to work independently, as would have been the case in almost every other laboratory at the time. This was not Calvin's idea of how a team ought to work together. Rather, Bassham immediately joined the laboratory's general agenda (he was to focus on degradation studies), and his subsequent publications, co-authored with many others, counted as the equivalent to the traditional single-authored thesis that usually qualified one for a doctoral degree.[55] One should add, though, that when Bassham started at the laboratory, in 1947, the photosynthesis group was still very small; indeed, it consisted only of Benson and Samuel Aronoff, another former doctoral student of Ruben's, who had spent two years at Chicago with Franck and Gaffron.[56] Aronoff's role at Berkeley was to take care of the algae and to develop appropriate culturing methods so that the state of the algae at least approximated a constant standard (no easy task, as was explained in Chapter IV of this book). Furthermore, because of his experience with plant material, Aronoff was in charge of ensuring that the biological side of photosynthesis did not go unheeded, since none of the chemists involved in the project knew very much about plants. (Note, however, that Aronoff's original field was not biology, but geology.)[57] Shortly after Bassham arrived, another graduate student, John Weigl, and two technicians, Tom Goodale and Gordon Hall, joined the project, taking the total number of members to five.

The one thing on which all group members agreed when recalling this period was the fact that the atmosphere in the clapboard building of the Old Rad Lab was special and intense in the early years of the project. The open-plan structure of the laboratory, which had only a few doors and compartments, strongly encouraged the continuous exchange of ideas and information among this group of young and talented scientists, who had gathered to solve the Big Mystery: the path of carbon in photosynthesis. (It is noteworthy that when the laboratory was founded, Calvin was not yet thirty-five and by far the oldest of the group!) Similar to the organisation of the original Rad Lab, the group was strongly focused on one single, if ambitious, research goal, to which all their energies were exclusively devoted. Calvin and Benson succeeded in creating an atmosphere of constructive tension and pioneering spirit, and it was this that made the group, which was very unusual in its range of disciplines, into such a closely collaborating unit. This is how Vivian

[54] Moses & Moses (2000), interview with Bassham, p. 7/2. On this episode, see also Bassham (2003), pp. 37f.

[55] Moses & Moses (2000), interview with Bassham, p. 7/4.

[56] On Aronoff's life and work, see, e.g., Govindjee (2010).

[57] See Moses & Moses (2000), interview with Bassham, p. 7/3.

Moses himself, who had come as a postdoctoral student from England, remembered these years:

> It soon became clear to me in the context of the lab that collaborations between people were highly encouraged. This was not something that I felt had happened in London [...] but was very much the name of the game in Berkeley. The pattern was that people would talk to one another – I was going to say, continuously [...]. They would say "Why don't we do so and so?". Faced with something that needed to be resolved, someone would say "Why don't we do this?" or "Why don't we do it that way?" and somebody else would join in and say "We could modify..." and so forth. And before very long you would find a new collaboration had been started. In addition to whatever it might have been that those people had been doing before, they added a new thing. This was continuously going on: people were constantly forming and re-forming collaborative associations. And, of course, it happened to me just as it happened to everybody else.[58]

Moses found that everyone was keenly interested in what everyone else was doing. The members of the laboratory was aware that the whole group was not only working in the same area but also working towards the same superordinate goal, the achievement of which would be to everybody's advantage. Of course, there was a need to stay informed, since otherwise one might miss important clues for one's own work. However the prevailing spirit was that collaboration not only paid off but was also fun and valuable in itself. The habit of openly discussing almost anything across the benches stayed vividly in the memory of many group members. "That was probably one of the most exciting of the learning experiences," the chemist Murray Goodman recalled forty years later. "You couldn't miss if you wanted to hear what was the latest, what was the conjecture, what was the abandoned hypothesis. It was all sort of put out there in a very open way for everybody to consider."[59]

The group's weekly seminars – held every Friday at 8 o'clock in the morning, thus, at a time at which many of the visiting scientists were not used to showing up at the laboratory, let alone ready to talk about science – were almost legendary. In the earliest days, the speaker was chosen spontaneously at the meeting itself. Calvin believed that everyone should be able to speak about his or her research at any time, so that he would just pick out a person from whom he had not heard any news recently. However, in view of the panic and distress that this practice caused among members of the group, after a year or so Benson persuaded Calvin that it would be more profitable if at least one day's notice was given (which usually resulted in the speaker staying up all night to prepare for the seminar). Rough treatment was par for the course; in fact, some participants were interrupted after just a few sentences and given no chance to continue, since Calvin would insist on exact information on every single point. As Rodney Quayle remembered: "Once you started with a seminar, Calvin could tear you to pieces. He got lost in the science, totally divorced from any personal feelings, and he would shred you. If the science was bad, he would be so carried up with it that he would shred you

[58] Moses & Moses (2000), interview with Moses, p. 17/8.
[59] Moses & Moses (2000), interview with Goodman, p. 14/4.

to bits."[60] Quayle once witnessed one of his fellow research students bursting into tears, because Calvin would not let go about some detail of her work; upon which Calvin looked terribly upset about what he had unintentionally done. It was only later, when the laboratory's group had grown larger in number that a formal schedule for the seminar was finally put together, and members would get a week's notice; in principle, though, the atmosphere remained the same.[61]

The size and diversity of the group were the salient features of Calvin's laboratory. Far from being only concerned with photosynthesis, Calvin also launched a project on chemical evolution and other themes that were only remotely related to the laboratory's core activities. While elsewhere rigid departmental structures still dominated (even within chemistry departments, the different subfields, such as organic chemistry or physical chemistry, were sharply separated from each other, with hardly any contact, not to speak of collaboration), in Calvin's laboratory all the boundaries were blurred. Quayle nicely recalled the contrast between the Calvin–Benson group and the other laboratories in which he had worked: "[S]uddenly to come into a lab where you were a scientist. [...] You happened to be a chemist, but the chap next to you was a botanist and the other chap next to you was a physicist, and if you hadn't ever seen a Warburg manometer in your life before, if it was necessary to use it, you learned."[62] Notwithstanding the divergent backgrounds and characters, a strong feeling of community existed within the group, which also included the administrative and technical staff. Calvin's long-time secretary Marilyn Taylor vividly remembered the atmosphere: "We felt like a family. Coming to work was like coming home. I think most people performed [well] in that kind of a situation. When necessary, you did 150% or 200% or whatever [...]. We all worked together as a group."[63]

In the next section of this chapter, I shall reconstruct the sequence of events that led the group working around Calvin and Benson to succeed in elucidating the photosynthetic carbon reduction cycle. For many years the latter was referred to as the "Calvin cycle"; however, in view of the decisive contributions that Calvin's most important collaborators made to its discovery, it would be more fitting to call it the "Calvin–Benson cycle" or the "Calvin–Benson–Bassham cycle", as is increasingly becoming the norm in photosynthesis circles today.

3 The Path of Carbon in Photosynthesis

3.1 The Standard Hypothesis and the First Cyclic Model (1948)

The line of research that Calvin and Benson decided to pursue once the laboratory had been set up was a direct continuation of the earlier experiments initiated by Ruben and Kamen: *Chlorella* and *Scenedesmus* algae were exposed for defined intervals to $^{14}CO_2$ (mostly administered in the form of a solution of sodium bicarbonate), after which the course of photosynthesis was stopped by the algae being killed (boiling alcohol was poured onto them), so that the sequence of intermediate products could be discerned. However, as Kamen and Ruben had

[60] Moses & Moses (2000), interview with Quayle p. 3/9.
[61] On the seminar's policy, see, e.g., Moses & Moses (2000), interview with Calvin, pp. 1/22–23.
[62] Moses & Moses (2000), interview with Quayle, p. 3/13.
[63] Moses & Moses (2000), interview with Taylor, p. 1/60.

found, this procedure, although easy to outline, was extremely complicated to realise in practice.

The principal assumption that guided the search for the mechanism for carbon reduction, not only at Berkeley but also elsewhere, was that photosynthesis was, chemically speaking, in many respects the reverse of respiration. This was not only suggested by the blunt fact that the products of the one process were the raw materials of the other, so that the principle of parsimony applied to this hypothesis. Assuming that the reaction steps were similar was additionally justified by the growing awareness that many biochemical reactions ran in both directions. Finally, comparative biochemistry was increasingly bringing to the fore the fact that many reaction mechanisms operated in exactly the same manner in plants, animals and bacteria. In a short note submitted to *Science* in 1938, the plant physiologist Kenneth V. Thimann, who had been Emerson's colleague at Caltech but had later moved to Harvard University (Cambridge, Massachusetts), succinctly summarised the starting point for any further study of the carbon reduction in photosynthesis:

> It is often stated that enzymatic processes are reversible. It is also a commonplace that photosynthesis is in many respects the reverse of respiration. Now there is every reason to believe that the CO_2 formed in oxidations arises from organic acids, probably by the same reactions as in fermentation, namely, from pyruvic acid by the action of carboxylase, from formic acid by the hydrogenlyase, from oxaloacetic acid in its breakdown to pyruvic acid, from acetoacetic acid in the acetone formation etc. What could be more natural than to suppose that in photosynthesis the absorption of carbon dioxide takes place in the reverse way, by combination with an aldehyde, or, more probably, with an organic acid to produce a new carboxyl group? Specifically, a probable reaction is the combination of CO_2 with pyruvic acid to produce oxaloacetic or perhaps with lactic acid to produce malic. The light reaction would then be the reduction, not of CO_2 as such, but of the carboxyl group.[64]

Thimann argued that, although it had been assumed since the time of Willstätter and Stoll that in photosynthesis carbon dioxide formed a complex with chlorophyll and was reduced in this complex by a light-driven reaction, no convincing evidence had ever been provided to support for this assumption. Even less evidence had been produced for the assumption that formaldehyde was the first reduction product; in fact, Thimann believed that "the persistence of these unsupported theories must be ascribed to the absence of any plausible substitute".[65] He found it far more convincing to think of photosynthesis as a process involving a cycle of the "combination of carbon dioxide with an organic acid, the photo-reduction of the carboxyl group and the consequent intramolecular changes leading finally to

[64] Thimann (1938), p. 506. Note that Thimann did not yet cite the paper of Krebs & Johnson (1937) in which the components mentioned by Thimann were knitted together into the metabolic cycle, which later became known as the tricarboxylic acid cycle or "Krebs cycle". However, Thimann did cite Krebs's work on the urea cycle, Krebs & Henseleit (1932), as the first instance in which evidence had been obtained that intermediate products act "as catalysts" in a metabolic cycle. See Nickelsen & Graßhoff (2008) for a discussion of the importance of this new concept of catalysis, which was proposed by Krebs in 1932.

[65] Thimann (1938), p. 506.

the setting free of the organic acid again".[66] As in other cases, some of the organic acids would be withdrawn from the cycle and reduced to sugar.[67]

Thimann's appeal that one should pursue this promising approach did not go unheeded; and the general idea – that there was a close parallel between the respiratory process and the process of photosynthesis – was the prevailing concept when the Calvin–Benson group started its work. By then, the biochemical pathway of cell respiration had been largely uncovered, including: (1) the process of glycolysis, that is, the degradation of glucose to pyruvic acid via the formation of phosphoglyceric acid and triose phosphates (phosphoglyceraldehyde and dihydroxyacetone in equilibrium); (2) the tricarboxylic acid cycle, that is, the stepwise oxidation of the carbon residue, whereby reducing equivalents are formed; and (3) the first ideas about how to formulate the final oxidation process, including the fact that cytochromes might be involved.[68] Furthermore, as mentioned earlier (p. 264) by 1940 it had become clear that carbon dioxide fixation in heterotrophic tissues was a very general phenomenon, and that it usually involved a partial reversal of the tricarboxylic acid cycle. The assumption that photosynthetic carbon dioxide reduction should proceed in a similar way seemed to be well supported by the only more or less positively established piece of data: the finding by Ruben and Kamen that the carbon dioxide in photosynthesis was fixed in a carboxylation reaction that supposedly yielded an aldehyde or a carboxylic acid as the first product. Modelling the path of carbon in photosynthesis along the lines of the path of carbon in non-photosynthetic fixation was, therefore, a well-founded standard hypothesis.[69] (As background information for later comparisons, the biochemical steps of glycolysis and the heterotrophic carbon dioxide fixation have been reconstructed in graph form in figures V.7 and V.8.)

Thus, when the Berkeley group started working on photosynthesis in 1946, they had a well-defined goal and a convincing working hypothesis of the mechanism in question. What they lacked, however, was the optimal means to pursue their goal. In their first attempts to clarify the whereabouts of the radioactively labelled carbon dioxide, to which the algae had been exposed, the group used classical

[66] Thimann (1938), p. 507. Note that Thimann still believed that the light reaction was connected to the reduction of carbon dioxide!

[67] Thimann (1938), p. 507.

[68] The decisive publication on the tricarboxylic acid cycle (also known as the "Krebs cycle") was Krebs & Johnson (1937); see Florkin & Stotz (1975), Chapter 33, and Holmes (1993) on the history of this discovery. A major breakthrough on the elucidation of the final electron transport chain was achieved in Keilin & Hartree (1939); for a historical review, see Florkin & Stotz (1975), Chapter 36.

[69] There is actually a second path of photosynthesis, called "C_4 photosynthesis" (since its pathway includes a number of C_4 organic acids), which partially diverges from the one investigated in Berkeley. The first indication of the existence of C_4 photosynthesis was found in the late 1950s (although the findings were published only years later). Its pathway does involve some of the intermediates of the tricarboxylic acid cycle, such as malate, oxaloacetate and pyruvate. On the discovery of C_4 photosynthesis see, e.g. Hatch (2002), which includes more references. The first publications on C_4 photosynthesis include Kortschak, Hartt & Burr (1965), Hatch & Slack (1966) and Hatch, Slack & Johnson (1967). Note, furthermore, that the occurrence of a "reductive carboxylic acid cycle", which in its overall effect was a tricarboxylic acid cycle run in reverse, was found in the 1960s, see, e.g., Evans, Buchanan & Arnon (1966), and the retrospective account by Buchanan & Arnon (1990).

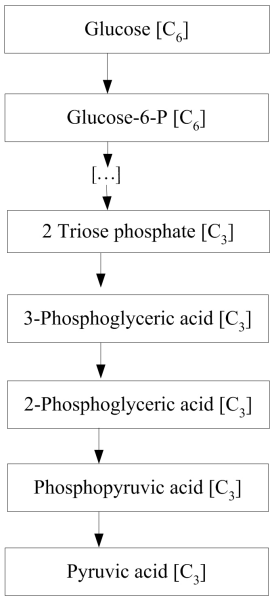

Figure V.7: The sequence of carbon compounds in glycolysis (degradation of glucose).

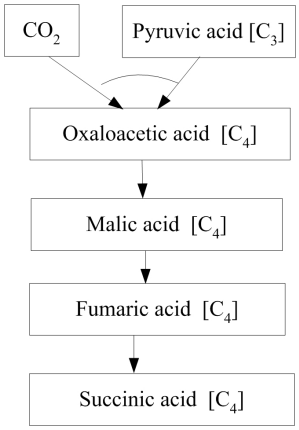

Figure V.8: What was held to the process of carbon dioxide fixation in heterotrophs in 1940. The sequence from pyruvic acid to succinic acid is a partial reversal of the tricarboxylic acid cycle in cell oxidation processes.

chemical procedures to try and identify the intermediate compounds from carbon dioxide to carbohydrates. However, they soon realised that these methods would not lead them anywhere: they were far too slow and required far too large amounts of plant material.[70] So the group changed over to using ion exchange columns.[71] The second major difficulty was ensuring that the labelled products (if they could be identified at all) were, in fact, products of photosynthesis and not the result of a non-photosynthetic carbon dioxide fixation. The Calvin–Benson group tried to avoid this difficulty by using periods of "pre-illumination": the cells were strongly illuminated in the absence of carbon dioxide for a period of at least ten minutes and sometimes for up to more than an hour, after which the light source was switched off and the algae were supplied with labelled carbon dioxide. The Berkeley team found that large amounts of carbon dioxide were almost immediately fixed, exceeding by far the usual dark fixation rate. They concluded from this that the increase in fixation was in fact due to the foregoing illumination period, during which the necessary amount of "reducing power" had been produced. Compared with the high rate of photosynthetic carbon dioxide fixation under these circumstances, the Calvin–Benson group thought that the risk of confounding photosynthetic with non-photosynthetic fixation products was negligible. (However, this procedure was soon criticised, in particular by members of the Chicago group, who found it less than reliable and were not at all convinced by the Berkeley team's conclusions. It was a problem comparable to the difficulties in differentiating manometrically between the gas exchanges caused by photosynthesis and those caused by respiration; a satisfactory solution was equally unattainable with the methods at hand.)[72]

The third complication concerned the inherent limits of tracer studies, which in the initial excitement had been overlooked by most researchers, yet in the course of time became more and more obvious. It transpired, for example, that since carboxylation reactions were reversible, all kinds of exchange reactions were occurring in the plant, so that not all the compounds, from which radioactively labelled carbon emerged, could be counted as being part of a carbon dioxide fixation chain, photosynthetic or not. Furthermore, it became apparent that in many metabolic pathways a clear preference existed for the use of ordinary carbon (^{12}C), so that far less radioactively labelled carbon was being incorporated into the plants than had theoretically been expected. How one should deal with these difficulties was far from clear.

However, despite all these challenges in experimental procedure, in 1947 the Berkeley team published a tentative report: after a period of illumination of five minutes, about 70 per cent of the labelled carbon was found in the succinic acid [C_4] and 3 per cent in the fumaric acid [C_4]; 15 per cent was present in a cationic fraction, presumably amino acids, while another 9 per cent of the

[70] See Calvin (1989), p. 9.

[71] According to Benson, this came about through Calvin's involvement as a consultant to Dow Chemicals, where new resins were being developed at the time. See Moses & Moses (2000), interview with Benson, p. 12/18.

[72] See Benson & Calvin (1947) and Calvin & Benson (1948) for the first publications from Berkeley; the approach was criticised in, e.g., Brown, Fager & Gaffron (1949), Fager (1949), Gaffron & Fager (1951).

products had anionic properties.[73] However, the list of labelled compounds after a period of illumination of thirty seconds, which was published in 1948, drastically differed from the first account: it was now found that most of the labelled carbon dioxide was incorporated into the malic acid [C_4], alanine [C_3], triose phosphate [C_3], phosphoglyceric acid [C_3], glucose [C_6] and fructose [C_6] as well as into the latter two compounds' phosphate esters. Remarkably, Calvin & Benson (1948) no longer found any intermediates of the tricarboxylic acid cycle among the early photosynthesis products, such as succinic acid or fumaric acid, which had been so prominently presented in the earlier paper.[74]

The 1948 list of products was complemented by the group's first impressions of the labelling patterns (that is, the temporal sequence in which the carbon atoms of a compound were labelled): in most of the acidic compounds, the labelled carbon appeared first in the terminal carboxyl groups. In the two hexoses, that is, glucose and fructose, the central carbons, at positions 3 and 4 in the chain, were the first to be labelled, while the labelling subsequently spread to the ends of the chain. The identification of large amounts of 3-phosphoglyceric acid (PGA) [C_3] and triose phosphate [C_3] with this particular labelling pattern was highly suggestive, because it was well-known that these compounds were among the first intermediates of glucose degradation in the course of glycolysis (see fig. V.7).[75] In fact, one might speculate that this was just what the Berkeley group had been hoping and looking for, bearing in mind that the standard working hypothesis of the photosynthesis model was that it was the reverse of respiration. From their data, Calvin and Benson were pretty much convinced that the synthesis of glucose was the reverse of the well-known sequence of glycolysis:

> In view of the presence of such large amounts of radioactive triose phosphate and phosphoglyceric acid in the very short photosynthetic experiments (30 sec) as well as in the dark fixation, it can be taken as fairly certain that the hexose synthesis proceeds by a reversal of the usual glycolytic split of fructose diphosphate, and therefore some path must be found by which the radioactivity appears first in the number 1 carbon atoms of the 3-carbon compounds and gradually spreads into the number 2 and then the number 3 carbon atom of the triose phosphate and the phosphoglyceric acid.[76]

In addition, Calvin and Benson suggested that this reaction was accompanied by a cyclic path, which would regenerate the carbon dioxide acceptor, and in which the various [C_4] dicarboxylic acids were involved:

> So far we have been able to account for the major fraction of the radioactive carboxylic acids formed in terms of only the 4-carbon acids. [...] We are therefore led to the supposition that the 3-carbon compounds are produced through the 4-carbon dicarboxylic acids. Furthermore, the acceptors for the one or more carboxylation reactions which take place must be regenerated not

[73] Benson & Calvin (1947), p. 648, Table 1. The abbreviation [C_4] denotes that this compound contains four carbon atoms, usually as a backbone chain. Within a compound the carbons are given numbers according to their position in the chain.

[74] Calvin & Benson (1948).

[75] See Calvin (1989), p. 9; Calvin (1962), pp. 880–881.

[76] Calvin & Benson (1948), pp. 478–479.

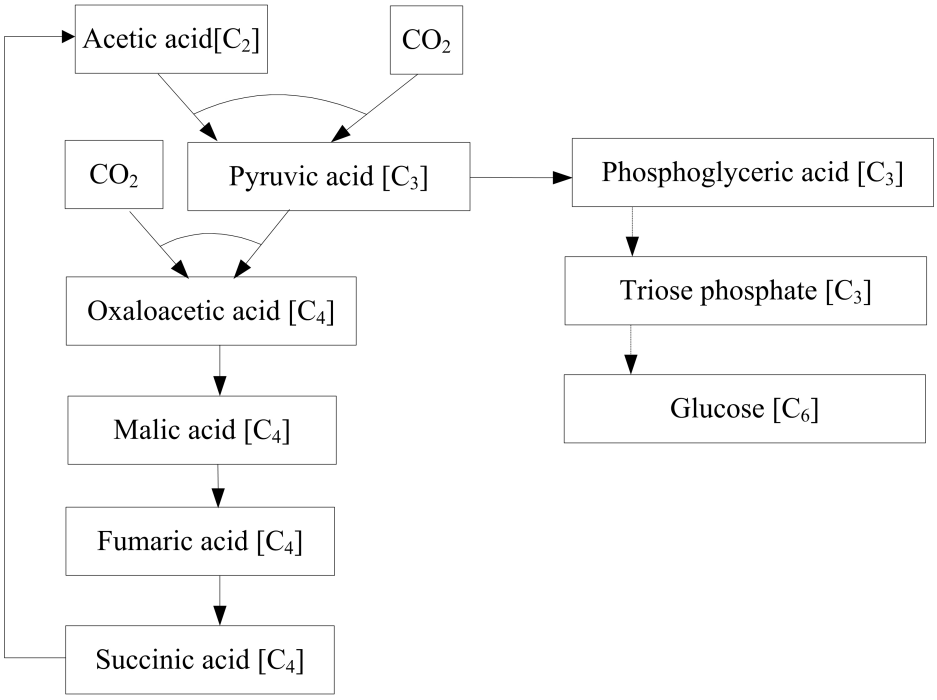

Figure V.9: The photosynthetic carbon cycle, as formulated by the Berkeley group in 1948. Note the parallels to the processes of glycolysis and to heterotrophic carbon dioxide fixation.

> only continuously but in such a manner as to contain a gradually increasing amount of isotopic carbon, relatively rapidly approaching the isotopic concentration of the carbon dioxide itself. All this can be achieved, with currently used reactions, only by the presence of what is effectively C_2-C_1 addition.[77]

Assuming the presence of a cyclic path was not a particularly original idea at the time – Thimann had already suggested something along these lines in 1938 (see quote on p. 275) – and presumably many others before and after him had the same idea. Metabolic cycles were omnipresent in the biochemistry of the 1930s and 1940s, and more and more of them were being elucidated. However, the general assumption now being endorsed by Calvin and Benson's finding was that there was a number of compounds that were being rapidly saturated with radioactivity (that is, in a very short time span all their carbon atoms were being radioactively labelled). Calvin and Benson's explanation was that these compounds were part of a cycle, which regenerated the original carbon dioxide acceptor and in which all the existing carbon atoms were quickly replaced.

The pathway, as formulated by Calvin and Benson in 1948, is reconstructed in the form of a causal graph in figure V.9.[78] The idea was rather simple: in line with the standard hypothesis, the fixation of carbon dioxide during photosynthesis was

[77] Calvin & Benson (1948), pp. 478–479.

[78] The same model hypothesis was still published in Benson, Calvin, Haas, Aronoff, Hall, Bassham & Weigl (1949), p. 399.

assumed to function in exactly the same way as the non-photosynthetic fixation of carbon dioxide, that is, by a reverse of the well-known decarboxylation reactions in the tricarboxylic acid cycle. Acetic acid [C_2] was thought to act as the first carbon dioxide acceptor, the carboxylation of which would lead to the formation of pyruvic acid [C_3]. The latter would be subject to a second carboxylation, which resulted in the formation of oxaloacetic acid [C_4]. Via the stages of malic acid [C_4], fumaric acid [C_4] and succinic acid [C_4], the cycle would be closed by the splitting of the latter into two molecules of acetic acid [C_2] again. Starting from pyruvic acid, the way was also open to the formation of glucose by a reversal of the steps of glycolysis. The energy required for all these reactions was thought to be provided by reducing equivalents, most of which were assumed to come from the photochemical parts of photosynthesis. This model not only had the advantage that it was exclusively based on reaction mechanisms, which were part of the standard biochemical body of knowledge, but it also neatly explained the sequence in which the radioactively labelled carbon appeared in the compounds, as Benson et al. emphasised in their contribution of 1949:

> It is clear from an examination of the cycle that, if it is running with tracer CO_2, the tracer will appear first in the carboxyl group of the three-and four-carbon acids, then, in the carboxyl group of the acetic acid and of the β-carbon atoms of the pyruvic acid. If the cycle should be stopped (the plant killed) after a relatively short period of operation, the specific activity of the carbon in each of the above named positions will be found to decrease in the order named. This is in accord with the available data [of the sequence of labelling patterns].[79]

This argument requires some qualification, though. However convincingly the cycle was able to explain the labelling pattern of the compounds, it had not been put together for this purpose. The same cycle had already been proposed in Benson & Calvin (1947), when the knowledge of the labelling pattern was still rudimentary and the only compounds assumed to be involved, with some probability, were succinic and fumaric acids. The rest was based on background knowledge, as the authors saw no reason to conceal, if one reads how they introduced their model in 1947: "Using some of the reactions already established in animal tissue and bacteria, it is possible to account for the above results as well as the observed distribution of radiocarbon in short photosynthesis."[80] The model was, in fact, nothing but the standard hypothesis fleshed out on paper, and up to 1949 the data fitted the model rather neatly.

3.2 Paper Chromatography

While the findings of the Calvin–Benson group reported so far were based on their work with ion exchange columns, the decisive secret of the group's sweeping success, in addition to using carbon-14, was their use of partition paper chromatography. This technique, developed in 1944 by a group of British chemists, became the principal analytical tool of the Berkeley team for identifying radioactively labelled

[79] Benson et al. (1949), p. 399.
[80] Benson & Calvin (1947), pp. 648–649.

products.[81] It all started in 1947, with the arrival at Berkeley of the graduate biology student William Stepka. He had become interested in using paper chromatography to separate amino acids while a student at Rochester University, New York, and he brought the technique with him when he moved to Berkeley to undertake his PhD studies. Although, at the time, paper chromatography was thoroughly despised by many biochemists and described as being mere "spots on paper", Benson and Calvin quickly grasped the enormous potential of the technique: it was not only much faster than ion exchange columns but also more precise.[82] Since Stepka was already an expert in amino acids, the Berkeley team first used these compounds when trying out the new separation method; however, the group very soon adapted the technique so that it could be used more broadly and with other compounds.[83]

The underlying principle was simple: from the concentrated extract of an algal suspension, containing all the potentially labelled compounds, a small drop was spotted onto a piece of filter paper. The overall radioactivity of this sample was quantitatively determined, and the paper was then hung in a closed chamber, with one of its edges (the edge below the spot of the sample) dipping into a trough comprising an appropriate solvent, such as water or ethanol. The solvent then moved up the paper by capillary action, met and dissolved the sample mixture and carried the compounds along, according to their solubility in the solvent. (The most soluble compounds travelled fastest up the paper.) The sample mixture separated out into different spots. The general structural properties of the various components could then be inferred according to their position on the paper. Having been eluted, the components of the spots could also be analysed by chemical or physical means. The Calvin–Benson group tried, for example, to apply fluorescence and ultraviolet absorption spectra to find out more about the compounds. However, most of the time there was not enough substance available for these analyses, so that the researchers had to use more traditional analytical techniques. The technique of co-chromatography, whereby substances assumed to have been in the spot were applied to the paper at the same time as the suspension to be analysed, was carried out in order, finally, to identify the substance: if the properties of the known molecule's chromatogram coincided with those of the substance in question, one could then conclude that the two of them were identical.[84]

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[81] See Consden, Gordon & Martin (1944) for the publication of the method; this was based on the seminal suggestion published in Martin & Synge (1941).

[82] See, e.g., Kamen (1985), p. 193. Note that, according to the plant physiologist Albert Frenkel, Stepka had to talk Calvin and Benson round to trying out paper chromatography to identify labelled intermediates; Frenkel also emphasised that it was Charles Dent who had brought paper chromatography from England to the US in the first place and who was instrumental in first attempting to identify ^{14}C-labelled amino acids. See Frenkel (1993), p. 106; among the early relevant publications were Dent, Stepka & Steward (1947*b*), Dent, Fink & Fink (1947*a*) and Fink & Fink (1948).

[83] See Stepka, Benson & Calvin (1948) for the first publication from the Berkeley group based on paper chromatography (on amino acid separation); Benson, Bassham, Calvin, Goodale, Haas & Stepka (1950) then demonstrated how successfully paper chromatography could be applied to identify carboxylic acids and phosphate esters.

[84] For a detailed description of this procedure, see also Calvin (1989), p. 9; Calvin (1962), p. 881; and Bassham & Calvin (1960), pp. 890–895.

However, although the principle was simple, the details were highly intricate. Several improvements had to be introduced before paper chromatography revealed its full potential. First, the team developed a second dimensional chromatography, in order to separate the labelled compounds more finely: after the first solvent had dried out, the paper was turned at right angles and submitted to a second chromatography run, with a different solvent. This strongly increased the chances that as many compounds as possible were separated from each other: two intermediates might have the same extent of solubility in a specific solvent, but it was unlikely that they would share the same extent in a completely different solvent. Second, paper chromatography was combined with autoradiography: since the compounds were radioactively labelled, their position on the paper could be visualised by placing an X-ray film onto the paper. This made it far easier to localise the spots and to preserve the chromatograms (since the paper was frequently destroyed at the analytical stage). For quantitative work, the amounts of radioactivity in the spots were determined with a Geiger counter. Third, the solvents themselves had to be adapted, since neither water nor ethanol nor any other standard solvent was able to separate the many phosphate compounds that by then were known to be contained in the extracts. It was chiefly Benson who developed this aspect of chromatography; he persistently experimented with one solvent after the other until he came up with a sophisticated variant that could also separate the sugar phosphates.[85]

Paper chromatography eventually became a highly powerful analytical tool. In the course of time, the Berkeley team succeeded in producing whole series of chromatograms from which the sequence of radioactively labelled compounds could be inferred (see fig. V.10 for an example of a chromatogram after different time periods). However, it was neither a straightforward, nor a very pleasant procedure to carry out: the solvents – highly noxious organic compounds – had an extremely strong odour, so that, following increasing complaints of the group members about the constant exposure to these dreadful organic vapours, the chromatography room was eventually moved out of the laboratory into an isolated spot. This, of course, did not change the situation for those carrying out the procedure. (Bassham recalled that some laboratory members who were working with the new solvents were once asked to leave a cinema because of complaints from other cinema goers seated nearby – they had shed their laboratory coats but otherwise not changed their clothes.[86]) Moses, who most of the time carried out the procedure, vividly remembered how much he detested the work:

> I was always smelly-ish, of course, and when you put the solvent in [the tank], it got smellier and then you closed the lid and left the papers to let the solvent travel across the paper for however many hours it took. [...] Then you had to take the papers out of the tanks in order to dry them [...]. And you then lifted up this sodden wet piece of paper, held in place by two or three paper clips, and delicately took it to a drying rack in some sort of oven, hood really, fume cupboard. During this process, you got the full force of the vapours in your face [...]. Every now and again, you would jerk the papers a bit too hard

[85] Moses & Moses (2000); Interview with Bassham, p. 7/10. See also Benson (2002*a*) and Bassham (2003).

[86] Bassham (2003), p. 41.

> and they would tear; of course, they were wet paper and wet paper has no tensile strength. And if you weren't careful, these papers would occasionally simply tear and rip off and fall on the floor and that would be the end of that one. But most of them survived and you then dangled them in this hood arrangement and left them, I suppose overnight, and eventually they would be dry. They never stopped smelling, incidentally; they were always stinking of the solvent but, of course, they were not so strongly smelling as when they were wet. [...] It was a most painful activity. [...] It was one of the most boring things I have ever done in my life; [...] it was awful. [...] Anyhow, we used to spend lots of time there counting these spots on chromatograms and it was on that, on the data from those measurements, that everything depended. And so it was very important to get it done.[87]

With the help of paper chromatography, it became apparent that even after just thirty seconds, the radioactively labelled carbon had become incorporated into a broad range of compounds, so that it became necessary to shorten the duration of the experiments even further – they would eventually be reduced to fractions of a second.

3.3 The Second Cyclic Model (1950)

By 1950, the one thing that had been established beyond any doubt was that PGA was a central compound in the early stages of photosynthetic carbon reduction. Although members of the Chicago group (working around Gaffron) had previously disputed this, they were quick to admit that their failure to discover PGA in their samples had been due to methodical deficiencies.[88] Indeed, by 1950, PGA appeared to be the only compound identified in tracer studies that was almost certainly involved in photosynthetic processes. Paper chromatography had impressively shown that in short-time photosynthesis (thirty seconds of illumination) 80 to 90 per cent of the radioactively labelled carbon was present in the PGA; and this was confirmed by the use of other techniques. The other potential intermediates that were still being debated included pyruvic, malic and glycolic acids, while most of the compounds, which had been assumed by the Berkeley team to be part of the regenerative cycle in 1948, had disappeared from the array of promising candidates. Not even the existence of triose phosphates, which were highly probable intermediates on the pathway from PGA to glucose phosphate, were confirmed to be present with sufficient reliability.[89]

In fact, the Berkeley team had to acknowledge that the labelling of the compounds of the regenerative cycle, namely succinic, fumaric, tartaric and malic acids, as well as other acids closely related to the tricarboxylic cycle that they had originally proposed had been an artefact. With hindsight it became clear that, up to approximately 1948, most of the radioactivity measurements had been significantly influenced by the high background radiation emitted during the runs of the cyclotron in the Crocker Laboratory next door. Bassham recalled this frustrating realisation: "Further work with more careful shielding of the Geiger counter

[87] Moses & Moses (2000), interview with Moses, pp. 17/12ff.

[88] See Fager & Rosenberg (1950), p. 618. This did not mean, however, that the relationship between the Berkeley and Chicago teams became more relaxed!

[89] See Gaffron & Fager (1951), p. 91.

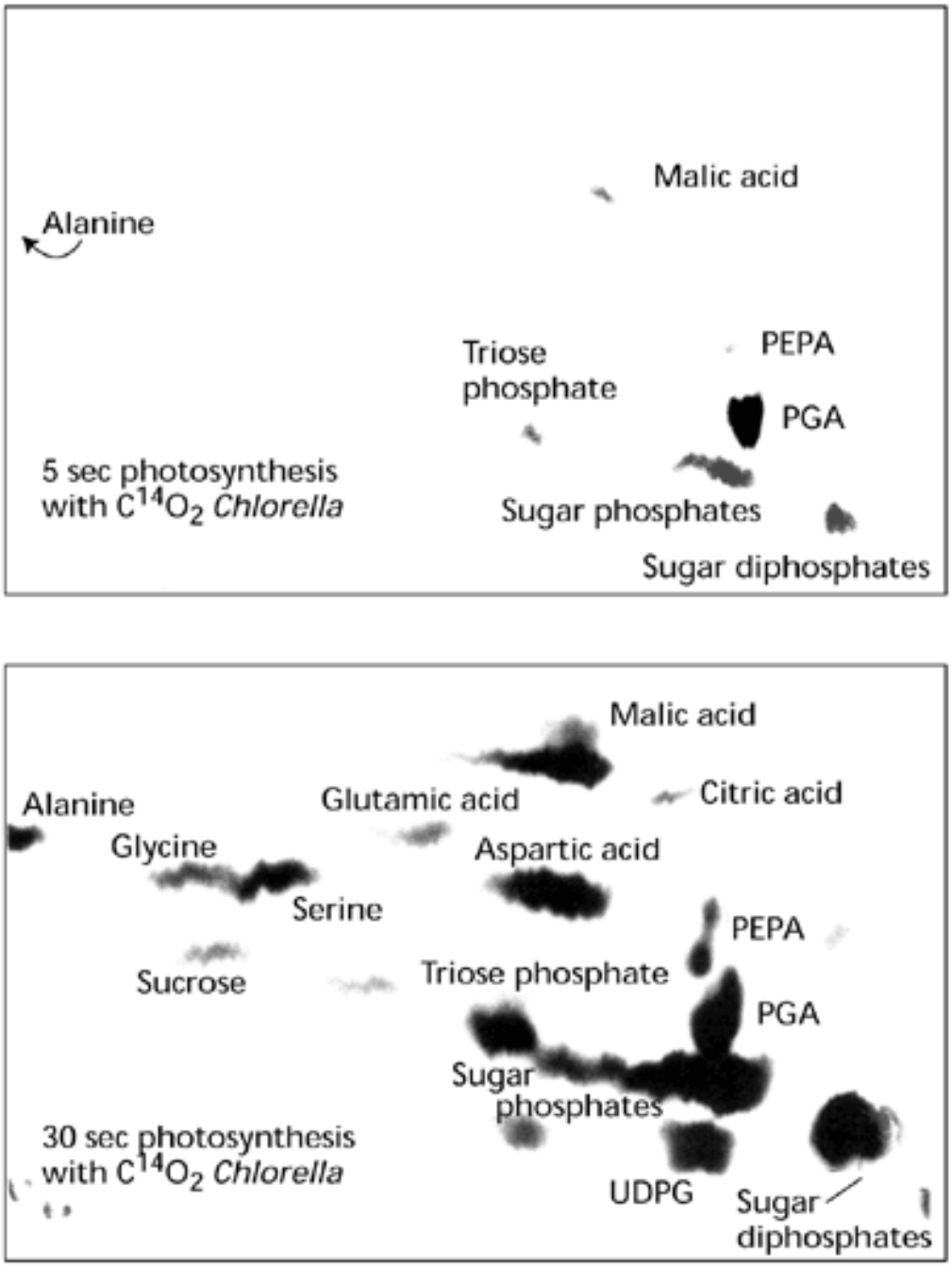

Figure V.10: A sample chromatogram, after five seconds (top) and thirty seconds (bottom) of carbon dioxide reduction. Note the rapid accumulation at the spot identified as PGA already after only five seconds.

showed that radioactivity was not spreading to the central positions fast enough for these compounds to be intermediates in the primary cycle."[90] The working hypothesis – that the process of carbon dioxide fixation during photosynthesis might operate in exactly the same way as the non-photosynthetic process – thus started to fall apart; the first uncritical wave of enthusiasm for the unlimited potentiality of tracer techniques also began to wear off. Emerson, for example, who had been delighted when he first heard about the experiments set up by Ruben and Kamen, now very much doubted the significance of the tracer studies' results, not least because there seemed to be no reliable method of differentiating between photosynthetic and non-photosynthetic carbon dioxide uptake. In a letter to Gaffron, written on 4 April 1950, Emerson maintained: "Provisionally, I conclude that pick-up of radioactivity, in either light or dark, is of doubtful significance as far as photosynthesis is concerned. It's going to be difficult to prove that any particular channel of pick-up is the channel of photosynthesis. I hear from California that radioactivity appears also in fats (??), 15 seconds after illumination."[91] Fats, of course, are large molecules that could not possibly have anything to do with the early stages of photosynthetic carbon dioxide reduction; and if substances like these were identified among the early products, how could one possibly rely on anything found using this means?

Nevertheless, the Berkeley group did not lose faith in their methods, and instead tried to learn from their mistakes. In 1950, they amended their proposal of the regenerative cycle, and now based it on the first extensive findings using the paper chromatographic methods (see fig. V.11 for a graphical reconstruction). In addition to the strong confirmation that PGA was one of the first products of photosynthesis, Benson & Calvin (1950) also endorsed the assumption that the hexoses might be formed from the PGA in a reversal of the process of glycolysis. Henceforth, the latter hypothesis on glucose formation was no longer seriously challenged. The new model was described by Benson and Calvin as follows:

> It is essentially a dicarboxylic acid cycle in which a two-carbon acceptor molecule is converted to oxaloacetate by two successive carboxylations. Upon splitting the four-carbon acid, two new acceptor molecules are formed. The intermediates of photosynthesis are diverted from this cycle for synthesis of fats, amino acids and carbohydrates.[92]

The 1950 cycle started with a hypothetical [C_2]-acceptor; the group suspected that acetic acid had this function but they had not yet been able to produce any evidence for this assumption. The acceptor was carboxylated (and phosphorylated) to form PGA [C_3]. (Going from this reaction step back to the acceptor's structure, the possibility was raised that the acceptor might alternatively be ethenyl phosphate, which, however, could also not be empirically substantiated.) Then PGA would either be supplied to form glucose-6-phosphate (in what constitutes the reversal of glycolysis), or it would be rearranged to form phosphopyruvic acid [C_3]. The latter was thought to be subject to the second carboxylation reaction, yielding

[90] Bassham (2003), p. 39.

[91] Emerson to Gaffron, 4 April 1950. Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Gaffron, Hans, University of Illinois Archives.

[92] Benson & Calvin (1950), p. 33.

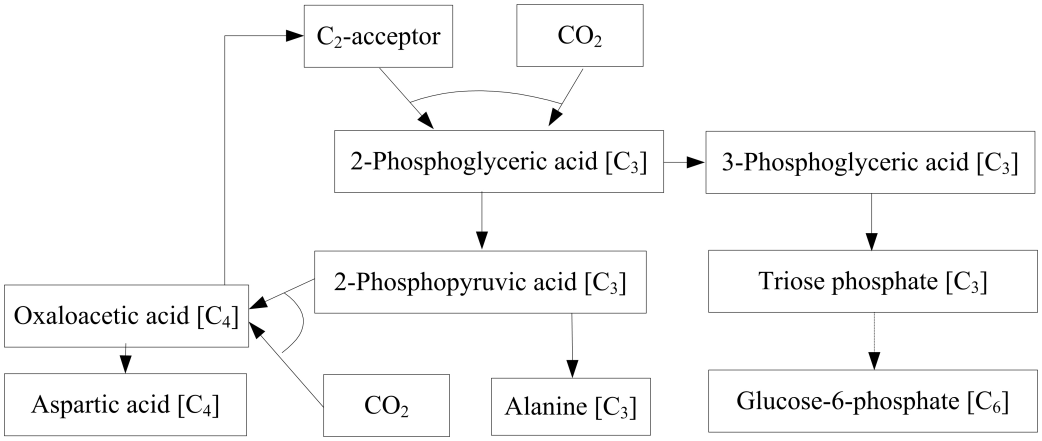

Figure V.11: The photosynthetic carbon cycle, as formulated by the Berkeley group in 1950.

oxaloacetic acid [C_4], which would regenerate the acceptor by splitting into two [C_2] fragments. Aspartic acid and alanine, two amino acids which were also among the early labelled products, could easily be formed from oxaloacetic or pyruvic acids.

The identity of the first carbon dioxide acceptor remained a mystery for some time. It was generally assumed to be a two-carbon compound [C_2] since the carboxylation of the latter was the easiest way to explain the formation of a molecule of PGA that had the radioactively labelled carbon incorporated into the carboxyl group. For some time, glycolic acid [C_2], which had been found to be present, was suspected to be closely related to the primary acceptor; however the data were unclear and the issue remained unsettled. Equally unclear was the *origin* of the hypothetical two-carbon acceptor, whatever its identity might be. A priori, two possibilities prevailed: either it was the result of a [C_1] + [C_1] combination or it was the outcome of the cleavage of larger compounds, for example, [C_4]. The Berkeley team consistently voted for the second option, not least because no one-carbon compound was ever identified in the chromatograms. In a paper of 1952, this decision was explained as follows:

> In order for it [the two-carbon primary acceptor] to result from the combination of two one-carbon fragments there must exist as an intermediate some one-carbon compound more reduced than carbon dioxide which, in turn, may combine either with itself or with carbon dioxide. Furthermore, the reservoir of this one-carbon intermediate would have to be vanishingly small since all attempts to find labeled, reduced, one-carbon compounds, such as formic acid or formaldehyde, in the early stages of photosynthesis have failed and, in addition, the resulting two-carbon fragment is very nearly equally labeled in both carbon atoms. [...]
>
> We are thus left with the following possibility for the C_2 compound – the cleavage of some C_4 or larger structure. The fact of the early appearance of label in malic acid, taken together with the lack of any appreciable amounts of label in the compounds of the tricarboxylic acid cycle, led us to the

> supposition that malic acid was either a precursor to, or very closely related to, a four-carbon compound which could be split to produce the required two-carbon fragment.[93]

This principal decision – to assume that the two-carbon acceptor was formed from the splitting of a four-carbon compound – explains why the Berkeley group was inclined to stick to the assumption of two consecutive carboxylation reactions. This assumption was supported by findings that seemed to indicate the existence, at least at low light intensities, of two independent reactions by which carbon dioxide was fixed: the carboxylation of the unknown two-carbon acceptor and the carboxylation of phosphopyruvic acid to malic acid.[94] (The latter finding seemed particularly significant: this was the pathway of the non-photosynthetic fixation of carbon dioxide, the enzyme for which had been found to be present in many plants.) However, very soon thereafter, malic acid was precluded from the list of possible intermediates, since inhibiting its formation did not significantly lower the amount of PGA produced.[95] Malic acid, the authors concluded, was not directly part of the regenerative cycle but rather formed from other participants, possibly oxaloacetic or another [C_4] acid.[96]

3.4 The Third Cyclic Model (1952)

The Berkeley perspective on the path of carbon completely changed when an important – and exciting – discovery was made under the leadership of Benson in 1951. Evidence was found for the existence of two entirely unexpected compounds among the products of short-time photosynthesis: sedoheptulose phosphate [C_7] and ribulose phosphate [C_5].[97] The finding of sedoheptulose phosphate, in particular, was hard to comprehend. In a retrospective account of the discovery, Benson recalled how these unusual compounds were first seen in a chromatogram of bacterial photosynthesis in *Rhodospirillum rubrum*, which had been prepared by Clinton Fuller:

> Their surprising appearance was tantalizing. After preparing hundreds of radiograms from our two-dimensional paper chromatograms, the usual pattern of compounds and their relative amounts had become very familiar. But, in this case, two radioactive spots just jumped out at us, strangers among a well known group of compounds. It must have been the result of phosphatase activities liberated in preparation of the bacterial extracts. I eluted our phosphate ester compounds and hydrolyzed them with Polidase [a fungal phosphatase]: the same two new compounds appeared, with the usual glucose, fructose, and triose. Soon it was clear that they were sugars, being neutral and hydroxylated. But they were neither hexoses nor known pentoses.

[93] Calvin & Massini (1952), p. 450.

[94] See Badin & Calvin (1950).

[95] See Bassham, Benson & Calvin (1950).

[96] The idea that the "malic" enzyme should somehow also be involved in photosynthetic carbon dioxide fixation was rather persistent. In Ochoa & Vishniac (1952), which was a very influential paper, the idea was not only revived but also expanded on, despite evidence to the contrary supplied by the inhibition studies.

[97] The first report on ribulose diphosphate was given in Benson (1951); the first report on sedoheptulose phosphate in Benson, Bassham & Calvin (1951).

> Frantically, I [co-]chromatographed one of the radioactive products with ribose, arabinose, and xylose: close, but not the same. The other unknown was even more confusing.[98]

With Bassham's help the compounds were finally identified. Bassham oxidised the unknown sugar with a reagent that would yield carbon dioxide from carbonyl carbon only, that is, from carbon that was double-bonded to oxygen, such as the carbon in position 1 of most sugars. To the team's utter surprise, only 14% of the carbon-14 in the originally eluted sugar was in this carbonyl carbon: that is, one seventh. "That's unheard of, Al, try again!," was Benson's first reaction to Bassham's finding. Bassham tried again, and confirmed his earlier result. Benson said later: "It was hard to believe – seven carbons!". They had to search the literature for any precedents of such a sugar, and really found that "sedoheptulose", a seven-carbon sugar, had been described in a publication of 1917.[99] The most recent paper on this topic had been written by the Norwegian biochemist Arnold Nordal. Benson wrote to Nordal and asked him for a sample of his sedoheptulose, on the basis of which the identity of the curious product was confirmed. An allegedly elusive compound, which had so far been reported to be present only in succulents, now seemed to perform a vital function in photosynthesis. It was found to be formed before the hexose phosphates, while it was too different in structure to be one of the latter's direct precursors. Thus, Benson assumed that sedoheptulose had a function somewhere in the regenerative cycle.[100]

The identification of the second mysterious compound was announced in the same issue of the *Journal of the American Chemical Society* in which the discovery of sedoheptulose was reported. Benson had managed to elucidate it through a laborious procedure. Step by step he succeeded in eliminating one improbable alternative after the other, until he concluded that the compound had to be (the equally improbable) ribulose diphosphate, which today is known as "ribulose bisphosphate". Furthermore, Benson had found that, under optimum conditions, the concentration of the ribulose diphosphate was similar to the concentration of PGA; and that the compound's degradation yielded PGA [C_3] and phosphoglycolic acid [C_2] as the two major products. Benson concluded his discovery report by announcing that "a discussion of its [the ribulose diphosphate's] importance as a C_2 donor in the cycle for regeneration of the CO_2 acceptors will be published".[101]

A major advance had been made, which was elaborated on in a publication of 1952 by Benson and a number of co-authors. Therein, they stressed the striking similarities between the five-carbon sugar and the seven-carbon sugar, and also speculated how the sugars might be related to the mysterious [C_2] primary acceptor of carbon dioxide:

> The close relationship between the structure of sedoheptulose and that of D-ribulose strongly suggests a synthetic relationship. The configuration of C-3 and C-4 of ribulose is identical with that of C-5 and C-6 of sedoheptulose. [...] None of these sugars is stereochemically related to glucose by a simple

[98] Benson (2002*a*), p. 39.
[99] Benson (2002*a*), p. 39. See also Bassham (2003), p. 42, for this episode.
[100] See Benson et al. (1951) for the pertinent publication.
[101] Benson (1951), p. 2972.

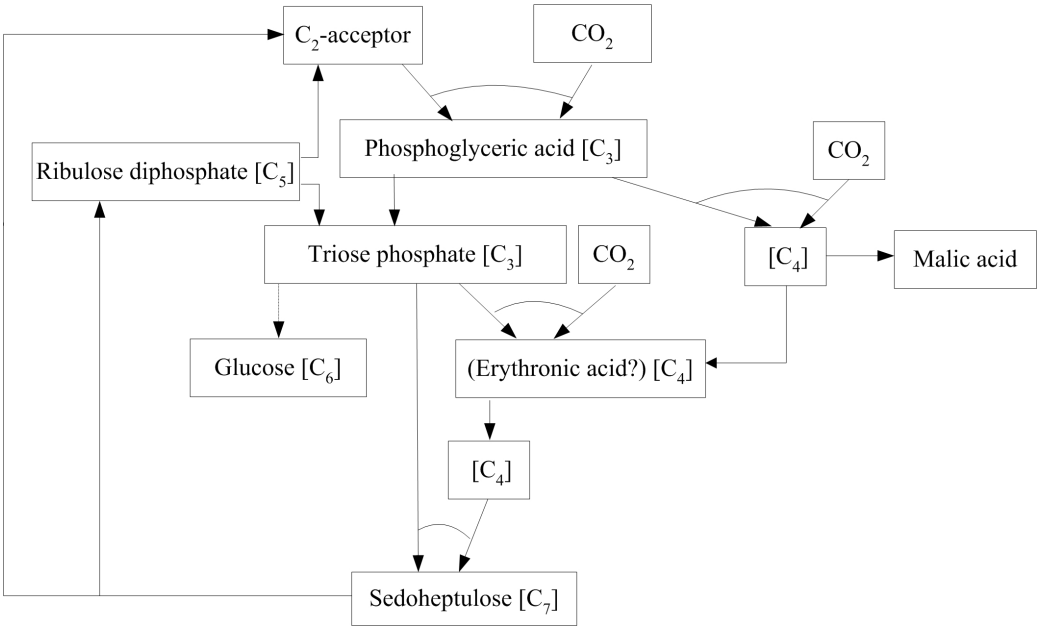

Figure V.12: The cyclic model of 1952. After Benson et al. (1952). (This is a simplified version; the potential links to other metabolic processes, such as the tricarboxylic acid cycle, have not been integrated into this reconstruction.)

> sequence of reactions. One of the functions of these compounds may be to serve as sources of 2-carbon molecules capable of accepting carbon dioxide to form phosphoglycerate during photosynthesis. The concurrent syntheses of fructose and sedoheptulose may represent steps in carbohydrate synthesis and in regeneration of the required carbon dioxide acceptors respectively. [...] The fact that the two predominant carboxylations of photosynthesis result in C_3 and C_4 compounds leads one to expect a condensation of the C_3 and C_4 sugars to give sedoheptulose.[102]

Around the same time, the Berkeley group had taken up extended kinetic studies of the radioactively labelled compounds. For this purpose, labelled bicarbonate solution was added at a certain point of time (t=0) to steady-state photosynthesising algae in which the mass concentration of the compounds was assumed to be constant. Thereafter, a series of samples was taken at certain intervals, and the appearance of the radioactively labelled carbon was plotted as a function of time. In addition to the general appearance, the distribution of radioactivity in each compound was also followed. The hope was that "[f]rom the nature of these appearance curves and the various compounds concerned it should be possible to determine their place in the complex synthetic system which must exist in the cell".[103]

[102] Benson, Bassham, Calvin, Hall, Hirsch, Kawaguchi, Lynch & Tolbert (1952*a*), p. 713.
[103] Benson, Kawaguchi, Hayes & Calvin (1952*b*), p. 4478.

One of the main results of these kinetic studies was that no appreciable reservoir of labelled carbon between carbon dioxide and PGA [C_3] was apparently present in the cell, which again indicated that PGA was one of the first products of photosynthesis. Furthermore, malic acid [C_4] was again found to be among the early labelled products; and while the inhibition experiments had precluded that it was part of the regenerative cycle itself, Benson et al. considered it likely that there was a four-carbon compound intermediate, possibly a derivative of erythronic acid [C_4], which was only present in very small amounts and which was in rapid equilibrium with malic acid.[104]

The appearance curves of the compounds, the Berkeley group argued in line with their previous suggestions, indicated that "there are two independent carboxylation reactions having different dependencies on carbon dioxide partial pressure."[105] The unknown four-carbon compound, the authors thought, was a likely candidate for the immediate precursor of the equally unknown two-carbon acceptor. Furthermore, it was found that fructose and sedoheptulose appeared to be labelled almost simultaneously (and rather early on), while the labelling of (newly synthesised) glucose and mannose lagged behind. In view of these findings, a new version of the photosynthetic cycle emerged, which is reconstructed in the form of a graph in figure V.12. As was quoted earlier, in this cycle the seven-carbon sugar was assumed to precede the five-carbon sugar as the latter's precursor. The authors conceded, though, that this hypothesis was still a tentative one, and that more evidence was needed.

In this new proposal of the cycle, the first step – from the [C_2] acceptor to PGA [C_3] – was unaltered; the PGA was then thought either to be rearranged to triose phosphate [C_3], upon which the latter was carboxylated and formed a [C_4], possibly erythronic acid, or, the PGA was carboxylated directly and formed an unknown [C_4] compound, which was in equilibrium with malic acid [C_4] and which was believed to be an alternative precursor of erythronic acid. The latter would give rise to yet another [C_4] compound, which, combined with triose phosphate [C_3], yielded sedoheptulose [C_7]. Then, sedoheptulose would be split into the unknown [C_2] acceptor and ribulose diphosphate [C_5], while the latter would be degraded as well and give rise to another [C_2] acceptor molecule and triose phosphate [C_3]. Finally, triose phosphate [C_3] was the usual starting point of the process of hexose formation, which constituted a reversal of glycolysis.

3.5 The Final Cyclic Model (1954)

The Degradation Studies

Although in the early 1950s, the time sequence and synthetic relationship of trioses and hexoses to PGA seemed clear enough, the same was definitely not true of the newly found five-carbon and seven-carbon sugars, despite the fact that a preliminary model had already been published (see the previous subsection). In fact, Calvin recalled that "attempts to establish this relationship by ordinary kinetic appearance curves of these two sugars resulted in conclusions on the order

[104] Malic acid is, in actual fact, one of the main intermediates in the alternative pathway of C_4 photosynthesis; see note and references on p. 276.

[105] Benson et al. (1952*b*), p. 4481.

of appearance of the pentose, hexose and heptose, which varied from day to day, experiment to experiment, and person to person".[106]

The person who was working most intensively with the sedoheptulose was the organic chemist Lorel Kay, who was a member of the Berkeley group from 1949 to 1954. Looking back on her work with a touch of self-irony, in an interview with Moses, Kay described herself as the world's expert, at the time, of sedoheptulose's middle carbon atoms.[107] Kay had to decompose the sugar, step by step, carbon atom by carbon atom, in order to identify the radioactively labelled carbons and the sequence of their appearance in the chain: a rather laborious procedure, since it transpired that, although the principal technique was well developed, each of the carbons in the seven-carbon chain had to be separated by a different method. Although Kay did not have to develop the methods herself, she had to go and find them in the literature and then implement the procedures, which might involve, for example, preparing the necessary enzymes from rats. Another challenge was to get hold of substantial amounts of unlabelled sedoheptulose to be used as carrier molecules. As no sedoheptulose was then commercially available, Kay and other laboratory members had to go out on a *Sedum* collecting expedition – luckily, satisfactory amounts of this plant were found near Calvin's home. Back in the laboratory, the sedoheptulose had to be retrieved from the leaves: "That was kind of a fun process, to do the mashing, like making wine perhaps," Kay remembered; "Mash it down, boil it down, eventually crystallise it and then I had my carrier to put in with the sedoheptulose that had the radioactivity in it from the short-term photosynthesis."[108] It was certainly not the kind of organic chemistry that was usually carried out in university laboratories.

As prolonged as the procedure was, as uncertain were the results. Although with hindsight finding the solution seemed entirely straightforward (how the sedoheptulose fitted into the path of carbon), in reality the process was, for a long time, only based on wishful thinking and the shakiest of empirical evidence: "Sometimes you'd get two [carbons] together and then you'd get one of another (sugar) and your data wasn't as neat and clean as perhaps you might have wished it to be," Kay remembered.[109] This was all the more annoying as Kay knew very well how closely her work was being followed by the others in the laboratory; she recalled how it was not always easy to defend her data:

> I remember that in some of the early days the data that I was getting didn't fit with the two CO_2 coming onto the recipient at the same time. So, there was almost, you know, a little pressure: "Hey, are you sure that this data is perfectly good?". I would say, "To the limits of how good I know it is, it says this and not that".[110]

As mentioned earlier, the Berkeley group had been convinced, up to this point, that there were two different carboxylation reactions in the cycle. Kay's data, however, did not provide any evidence to support this assumption; the latter was, in fact,

[106] Calvin (1964), p. 630.
[107] Moses & Moses (2000); interview with Kay, p. 20/5.
[108] Moses & Moses (2000); interview with Kay, p. 20/6.
[109] Moses & Moses (2000); interview with Kay, p. 20/5.
[110] Moses & Moses (2000); interview with Kay, p. 20/6.

irreconcilable with her findings. She recalled that Calvin and others tried to talk her out of it; only because she refused to give in, did the others finally capitulate.[111]

While Kay was the seven-carbon sugar expert, Anne Tolbert (at the time, Anne Harris) became the expert in five-carbon sugars. She joined the laboratory in 1951 as a graduate student; originally she had planned to become a high school teacher, but her college professor had strongly recommended that Tolbert do graduate work with Calvin. To Tolbert's utter surprise, she was accepted. When being interviewed by Moses, Tolbert reminisced that when she arrived at the Berkeley laboratory she did not know anything about photosynthesis or chromatograms – in fact, she did not know much about chemistry in general. However, Tolbert amply justified her professor's expectations. She quickly caught up and soon became truly engrossed for her research project, which turned out more challenging, more interesting and far more important than anybody had expected. It was assumed that, according to the model hypothesis favoured at that time, the labelling pattern of the ribulose would exactly match that of the sedoheptulose (since the latter was thought to be the precursor of the former). Tolbert said in retrospect: "They thought it was just some simple kind of mechanism where you just added two and took off two; I don't remember the story [...]. But I remember we were going to come out with the same kind of results. That was assigned to me because they knew what they wanted done."[112] Thus, to the people in the laboratory, presumably first Benson (since he was directly responsible), the project given to Tolbert was a piece of standard work that was deemed to be appropriate to her skills, and of which the outcome was pretty much determined from the start. Yet, the data came out rather unexpectedly, and, like Kay, Tolbert also had to face the fact that her findings did not meet the group's expectations. This is how Tolbert recalled her colleagues' reactions when the data disagreed with the working hypothesis:

> The idea was to make compounds that you could then break apart and then count those and then make another kind of degradation where you counted – there'd be other atoms and [you] just kept adding and subtracting. Every time we would finish doing this, we weren't the same at all [i.e. Tolbert and Kay did not obtain the same labelling patterns]. That's what I remember. I didn't have much faith in what I was doing because I didn't know how to do it; [...] So when these didn't come out, I thought, well I didn't do it right. I remember a staff meeting we had where we presented the data, my counts and her counts [...]. It was one of the seminar sessions where I've just had to present this, you know, and everybody was really kind of uptight because these weren't the same [the data for the labelling of the five-carbon and the seven-carbon sugar]. There was not this 1:1 correlation. It was really hard for me to defend this but I said, "Well, I think I did it right". "This is the way I did it" and I told everybody. As it turned out, it was, I think, correct, and it was a more complicated cycle than they thought.[113]

Although to a certain extent Kay and Tolbert had to fight their corner, Tolbert generally felt that the group, Calvin included, took her arguments seriously in the end. This was by no means a matter of course at the time, given the fact that they

[111] Moses & Moses (2000); interview with ay, p. 20/6.
[112] Moses & Moses (2000); interview with Tolbert, pp. 28/2–3.
[113] Interview with Tolbert, p. 28/3.

were both women and, furthermore, Tolbert was only a graduate student. "If you had something to say, they would listen," Kay recalled.[114] This general attitude of the group was confirmed by others, such as Quayle: "Calvin, himself, would learn from the man who cleaned the lavatories. If the man who cleaned the lavatories had by any chance a good idea, Calvin would have it out from him without a trace of embarrassment."[115] When Tolbert presented her work, and no obvious methodical mistake could be uncovered, the other members of the group sat down and thought about what this would mean – whether the cycle was, in actual fact, running differently to expectations. Tolbert stayed in the laboratory until 1953, and thus had the satisfaction of seeing how her data provided important clues to the eventual solution. However, by then she had married another graduate student, and when he got a job at Harvard, she took her Master's degree, instead of a PhD, and left Berkeley and science – which she clearly regretted.[116]

The problem was not only that the labelling patterns of the five-carbon sugar and the seven-carbon sugar disagreed but they did so in such a strange way (see fig. V.13). In the ribulose diphosphate, the central carbon atom (No. 3) was labelled first, followed by carbons 1 and 2; while in the sedoheptulose monophosphate the three central carbon atoms (3, 4 and 5) were the first to be labelled, with a slight tendency for number 4 to be labelled first. Interpreting these patterns was not straightforward. There were three possible ways of forming the seven-carbon compound: $[C_6] + [C_1]$, $[C_5] + [C_2]$ or $[C_4] + [C_3]$. The presence of PGA obviously provided a suitable $[C_3]$, so that the latter of these three options appeared the most promising at first glance. However, the labelling pattern of the three carbon atoms of PGA did not match either the top or the bottom positions of the sedoheptulose. The pattern of the ribulose diphosphate could not be detected in the sedoheptulose either, so that here too a direct precursor-product relationship seemed doubtful. To find an alternative origin of the ribulose was equally puzzling. A priori several options were possible: it could be formed either from a hexose losing a carbon fragment, that is, $[C_6] - [C_1]$, or from smaller fragments combining with each other, such as $[C_1] + [C_4]$ or $[C_2] + [C_3]$. No hexose with a labelling pattern approximately equal to that of the ribulose was found, so that the first option was ruled out. Again, the PGA easily provided the $[C_3]$ fragment, and the labelling seemed sufficiently similar. However, finding an appropriate $[C_2]$ remained problematic.

The Saturation Experiments

Parallel to the degradation work on the new compounds, a new type of experiment was devised in order to study the course of the pathway from a different angle. The capacity of the cycle's intermediate substances to become rapidly saturated with radioactivity was a useful starting point for determining the size of the reservoirs of these compounds and the way the system responded (by changes to the pool size of the compounds) to the variation of external variables, such as light.[117] The

[114] Interview with Kay, p. 20/5.

[115] Moses & Moses (2000), interview with Quayle, p. 3/13.

[116] See Moses & Moses (2000), interview with Tolbert, p. 28/7. In a later section of the interview (p. 28/9), Tolbert described how she took a long career break to raise a family and then eventually became an accountant.

[117] See Calvin & Massini (1952), p. 451.

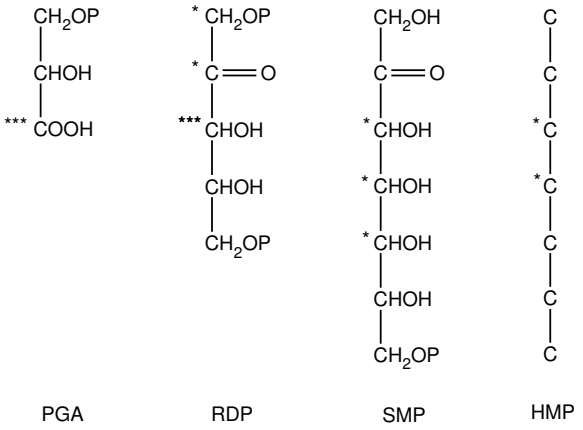

Figure V.13: The labelling patterns of phosphoglyceric acid (PGA), ribulose diphosphate (RDP), sedoheptulose monophosphate (SMP) and hexose monophosphate (HMP). After Bassham et al. (1954), p. 1767.

first of these experiments was carried out by the Swiss biochemist Peter Massini, who had come to the Berkeley laboratory in 1951 on an exchange visit for a year. This is how he remembered the experimental set-up for determining the pool sizes:

> I labelled the material for a certain time, a rather long time for what then was usual: a minute or several minutes. Then I switched off the light. Before and after switching off the light, I made [took] samples, from the lollipop, and made the chromatograms and everything. These compounds, first compounds were then in a state where the labelling was all already at [a] maximum. What happened after switching off the light was an index for the concentration of these compounds, the changes of concentration of the compounds other than changes of the specific activity. That was the point.[118]

Thus, the idea was to observe the accumulation or depletion of compounds known to be involved in the pathway by cutting one of the pathway's sources. In this case, the source to be cut was the energy equivalents gathered from the photochemical reactions. Massini remembered that the whole project was of great interest to Calvin and that the two of them exchanged news on the chromatograms on a daily basis. What he found was rather exciting:

> When illumination is interrupted there appears a sudden great increase in the concentration of phosphoglyceric acid (followed by a slow decrease after 2 min), and an almost complete depletion of the diphosphate area [made up almost exclusively of ribulose diphosphate]. Analysis of the monophosphate area showed that the amount of sedoheptulose phosphate decreased also.[119]

[118] Moses & Moses (2000); interview with Massini, p. 48/2. In this oral interview with Massini, his use of "switching out" the light has been corrected to "switching off" the light.

[119] Calvin & Massini (1952), p. 454.

In other words, when illumination was stopped two directly opposed responses were observed, that went in opposite directions: the concentration of PGA went up, while, correspondingly, the concentration of ribulose diphosphate and sedoheptulose monophosphate went down. This seemed to indicate that the three compounds were connected to each other in a reaction series: while the *formation* of PGA seemed to be independent of the light, the further processing of PGA "downstream" to ribulose diphosphate and sedoheptulose monophosphate was dependent on the supply of energy provided by the photochemical reactions. Furthermore, the accumulation of one compound and the depletion of others were just the kind of effects one would expect of a cycle. And although everybody had for long suspected that there was a cycle, it was only from these experiments that the concept of a regenerative cycle was given sound empirical foundation.

The light experiment was complemented by a study of what happened when the supply of carbon dioxide to steady-state photosynthesising algae was reduced. This became the project of Alexander T. Wilson, a twenty-year-old graduate student from New Zealand, who, supported by a Fulbright Fellowship, worked in the Berkeley laboratory from 1952 to 1953. Wilson remembered that, when he inquired with Calvin what he was to do, Calvin said: "Why don't you just work in the lab and help the other people and eventually we'll find something, we'll stumble across something which will make a good thesis." And so they did. In the end, it was Wilson's interest in instrumentation that earned him a pivotal role in the carbon dioxide saturation experiments that became so famous.

Wilson's experiments were done in parallel to Kay's and Tolbert's degradation studies, although, according to Wilson, the two lines of research exemplified totally different approaches to the problem: "They were trying to figure out the path of carbon to see where the carbons were in the individual five-, six- and seven-sugars and I was really on a different track altogether. I was trying to do it some other way, by building a fancy piece of apparatus and stopping the CO_2 and seeing what built up."[120] Rather than looking at the chromatograms as a static unit and trying to establish the linear sequence of events, Wilson wanted to acquire a more dynamic understanding of the process, which included analysing the interaction of all the components. In order to get there, he created a highly intricate experimental set-up, the "algal steady-state apparatus", as it became called, which allowed Wilson to keep the algae in a controlled physiological state at low temperatures. This slowed down the enzymatic reactions, so that Wilson was able to collect series of samples in order to study very short exposure times (0.4 to 15 seconds) of the plant to $^{14}CO_2$. Wilson explained the purpose of the complicated apparatus in his published paper as follows:

> Even when every attempt is made to control conditions under which algae are grown, the algae show daily variations in such properties as rates of CO_2 fixation and cell division. These considerations make it difficult to do experiments in which the results from different days must be compared on a quantitative basis. To overcome this difficulty the apparatus was designed to take small representative samples of algal suspension over short-time intervals from a system in which the external variables were under complete control.

[120] Moses & Moses (2000); interview with Wilson, p. 13/15.

> Use was made of recent advances in instrumentation to monitor continuously the variables, such as partial pressure of CO_2 and radioactivity.[121]

The "recent advances in instrumentation" referred to an infrared gas analyser and a corresponding recorder that continuously monitored the carbon dioxide concentration in the tanks. Before any samples could be gathered, Wilson had to cool down the whole set-up to a temperature of 6 °C, which was the only way to get sufficiently fine-grained data on the reactions. Since one could not speed up the measuring process indefinitely, Wilson tried, alternatively, to slow down the reactions by having them run at a lower temperature. Samples were taken at short intervals, going from 1 per cent to 0.03 per cent carbon dioxide and then in reverse. It was an ingenious apparatus, but it was impossible to run the series without the help of others. "I would set up the experiment and then everybody in the lab would help me for 30 minutes while I took all these measurements on the samples," Wilson remembered.[122] The effort proved worthwhile, since the data turned out to be extremely rewarding. This is how Wilson formulated the main result in his final publication:

> Perhaps the most striking result is the reciprocal relationship between PGA and RuDP [= ribulose diphosphate]. [...] As soon as the CO_2 pressure is dropped the PGA drops sharply and the RuDP rises sharply. The initial slopes of these curves, together with the fact that the other intermediates change more slowly, confirm that PGA and RuDP are related in a precursor-product relationship [...]. The results imply that RuDP is the actual CO_2 acceptor in photosynthesis, or alternatively is related to it by a vanishingly small reservoir, and that PGA is the first observable product of the carboxylation.[123]

This was the solution that finally brought to an end the relentless hunt for the two-carbon acceptor: there was no two-carbon acceptor. The substance was a five-carbon acceptor, ribulose diphosphate, which, immediately after carboxylation was split into two halves, yielding two three-carbon molecules of PGA – a triumph of parsimony, after all. The remarkable thing was that this process had no known precedents in organic chemistry. Nature, once more, had come up with a solution that chemists up to then had been unable to imagine. The whole experiment became a landmark in tracer studies, and is still remembered by some as "one of the great classics in dynamic biochemistry".[124] It was thanks to these experiments, carried out by Wilson and Massini, in combination with the results of the degradation studies, that the idea of a second carboxylation (leading to a four-carbon compound related to malic acid) was dropped. Wilson remembered that Calvin was one of the last to be convinced that the cycle really comprised only one carboxylation reaction.[125]

Although Wilson's findings were used in the famous "Path of Carbon XXI" paper of 1954, dealt with in the next subsection, a separate paper, published in

[121] Wilson & Calvin (1955), p. 5949.
[122] Moses & Moses (2000); interview with Wilson, p. 13/7.
[123] Wilson & Calvin (1955), p. 5952.
[124] Moses & Moses (2000); interview with Quayle, p. 3/5.
[125] See Moses & Moses (2000); interview with Wilson, p. 13/7.

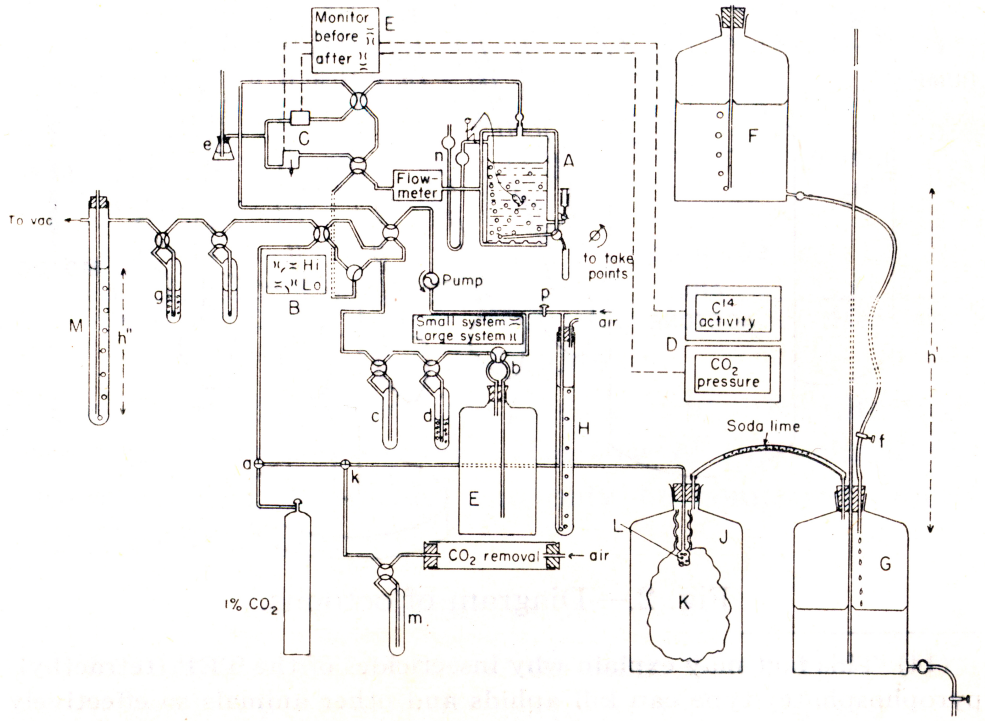

Fig. 1.—Diagram of apparatus for measuring transient phenomena.

Figure V.14: A drawing of Alexander T. Wilson's apparatus.

1955 in the *Journal of the American Chemical Society* (JACS), gave more details on the actual technique.[126] Among other things, the paper contained a detailed illustration of the highly complex apparatus that Wilson had constructed – perhaps one of the most complicated drawings ever published in the journal. Fuller recalled that several members of the laboratory had unsuccessfully tried to talk Calvin out of having this "unintelligible masterpiece" published in its entirety without being simplified.[127] The drawing was prepared by Alice Holtham (later Alice Lauber), one of the secretaries, whose office was next door to Wilson's apparatus; thus, she knew the set-up very well and had even been involved, at times, in taking measurements.[128] She remembered that completing this drawing exhausted her, and she was devastated when she learned that the journal's editors found it too big and wanted to compress it to the width of a text column.

Encouraged by some members of staff, including Wilson, Holtham spontaneously drew a tiny fisherman perched with his rod on a tube leading into the algal steady-state reservoir, and converted one of the bubbles in the reservoir into a fish. Her colleagues found this "add-on" hilarious, and urged Holtham to keep

[126] See Wilson & Calvin (1955).
[127] Fuller (1999), p. 8.
[128] See Moses & Moses (2000); interview with Holtham, p. 23/8.

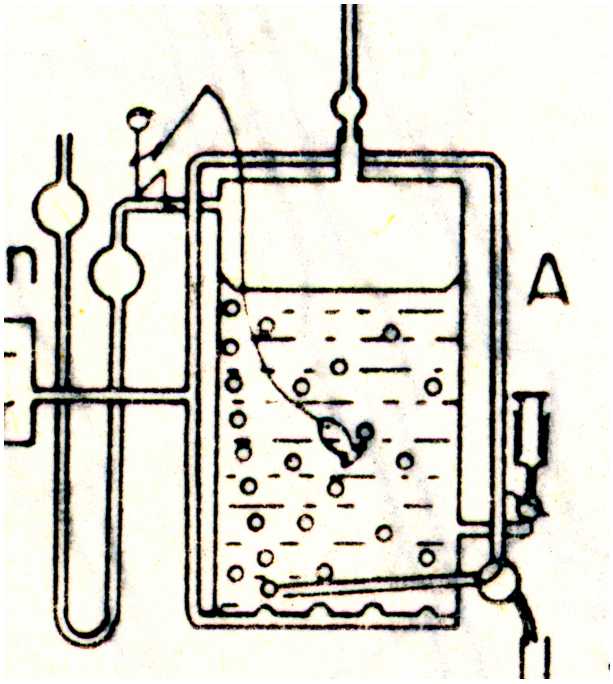

Figure V.15: Detail of Wilson's apparatus: the fisherman perched on a tube leading into the steady-state reservoir.

the drawing as it was when the paper was submitted to the journal, which she did. In its reduced form the illustration came out so small that the fisherman was hardly discernible, yet it was infallibly there. As none of the reviewers complained about it the drawing was published in its entirety by the JACS (perhaps the only humorous item ever to be published therein!), and it can still be found in most university libraries.[129]

The Path XXI Paper

The central paper in which the "definitive" model hypothesis, based on the data gathered in the saturation experiments and in the degradation studies, was presented, was published in 1954 and according to its number in the "path of carbon" series, it became known, within the Berkeley group, as the paper "Path XXI" paper.[130] A rough schema of the regenerative cycle, as formulated in this paper, is depicted in figure V.16. Ribulose diphosphate, the five-carbon sugar, was the acceptor of carbon dioxide, while the resulting six-carbon compound was highly unstable and immediately split into two molecules of PGA, or, alternatively, one molecule of PGA and one of phosphoglyceraldehyde. The further processing of the

[129] The story is well remembered by many members of the laboratory. See Moses & Moses (2000), e.g., the interviews with Calvin, Holtham, Kay, Moses, Wilson etc. See also Fuller (1999), pp. 8–9.

[130] See Bassham, Benson, Kay, Harris, Wilson & Calvin (1954). Bassham mentioned in his interview with Moses that, although the group had had a "pretty good handle on the cycle" before 1954, he always regarded "Path XXI" as being the definitive publication. See Moses & Moses (2000), interview with Bassham, p. 7/10.

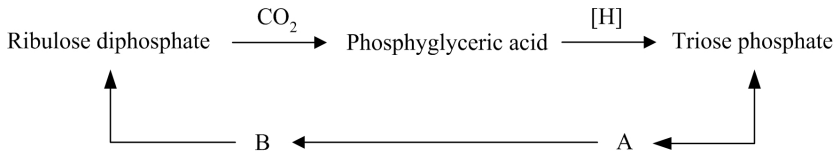

Figure V.16: Rough schema of the photosynthetic cycle. After Bassham et al. (1954), p. 1766.

PGA required an energy input from the photochemical reactions (in the form of reducing equivalents) and resulted in the formation of triose phosphate. The latter then became the starting point both of the formation of hexose phosphates and of the cyclic regeneration of ribulose diphosphate.

They then needed to match this schema with the labelling patterns that had emerged from the degradation studies. These data precluded the possibility that the ribulose diphosphate was entirely derived from a $[C_6] \rightarrow [C_1] + [C_5]$ split or a $[C_7] \rightarrow [C_2] + [C_5]$ split. "No five-carbon fragment of the hexose or the heptose molecules contains the same distribution of radiocarbon as ribulose" was the summarising statement, which in effect brought to an end this part of the 1952 model.[131] The third obvious option – a three-carbon compound combined with a labelled two-carbon fragment – was discarded as well: the only way to obtain an appropriately labelled two-carbon fragment was through the breakdown of the labelled hexoses – the actual occurrence of such a breakdown, however, was considered highly unlikely. The solution that was finally arrived at was a very counterintuitive one; and it was only when all the more promising alternatives had been ruled out that the Berkeley group came up with it. This is how it was introduced in the paper, which was authored by Bassham, Benson, Kay, Harris (later: Tolbert), Wilson and Calvin:

> Another way of accounting for the observed distribution of radioactivity, which seems quite plausible in view of the rapidly accumulating enzymatic evidence for the reverse reaction, is the formation of ribulose from sedoheptulose and triose. This reaction could result in the observed labeling. If the ribose-5-phosphate and ribulose-5-phosphate are then converted to RDP [ribulose diphosphate] the resulting distribution of labels would be that observed.[132]

Thus, the explanation for the confusing labelling patterns was that there were two different pathways that led to the formation of ribulose diphosphate: the first was when the two-carbon fragment from the top of the sedoheptulose was combined with triose phosphate; the second was the rearrangement of the remaining five-carbon fragment of the former sedoheptulose (see fig. V.17). At this point, the Berkeley team only had evidence for the occurrence of the reverse reaction (see quotation above), but it was known that most enzymatic reactions are reversible, which, to some extent, justified their postulate.

[131] Bassham et al. (1954), p. 1767.
[132] Bassham et al. (1954), p. 1767.

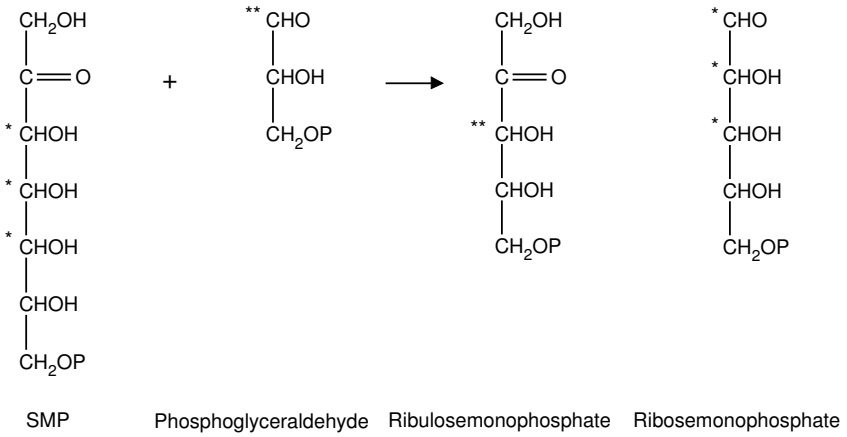

Figure V.17: The proposed mechanism for the formation of ribulose diphosphate: sedoheptulose monophosphate combines with triose phosphate to form two pentoses that can easily be converted into ribulose diphosphate. After Bassham et al. (1954), p. 1767.

The next question to be solved was the point of origin of the sedoheptulose. The degradation studies had ruled out the two obvious options – that it was formed by a $[C_6] + [C_1]$ or a $[C_5] + [C_2]$ reaction. There was still the possibility that a $[C_4] + [C_3]$ pathway was constructed; however, it was clear from the labelling patterns that the reaction assumed in 1952 (the formation of a $[C_4]$ via the carboxylation of triose phosphate) was incompatible with the data as Kay and Tolbert eventually persuaded Calvin and Benson. Therefore, by 1954 the Berkeley group favoured a different pathway:

> The most likely source of the $[C_4]$ fragment seems to be a $[C_6] \rightarrow [C_2] + [C_4]$ split. Trioses $[C_3]$ could then react with $[C_4]$ and $[C_2]$ to give sedoheptulose and ribulose, respectively.[133]

One possible formulation of these reactions would be the transfer of the top two carbon atoms of a hexose to one molecule of phosphoglyceraldehyde, which would yield one molecule of ribulose monophosphate and a $[C_4]$ fragment. The latter could then, in a second step, react with dihydroxyacetone phosphate to yield sedoheptulose monophosphate. This was a mechanism that very nicely fitted the labelling patterns (see fig. V.18). Phosphoglyceraldehyde and dihydroxyacetone phosphate, both of which are usually grouped together as "triose phosphates", were known to be in equilibrium with one another. The resulting sequence of biochemical steps is outlined in figure V.19. Three pentoses combined with three molecules of carbon dioxide would react to give six molecules of PGA, which then, supplied with 12 reducing equivalents, yielded six trioses. From two of these trioses a hexose would be formed, which, when combined with another two trioses, would react to

[133] Bassham et al. (1954), p. 1767.

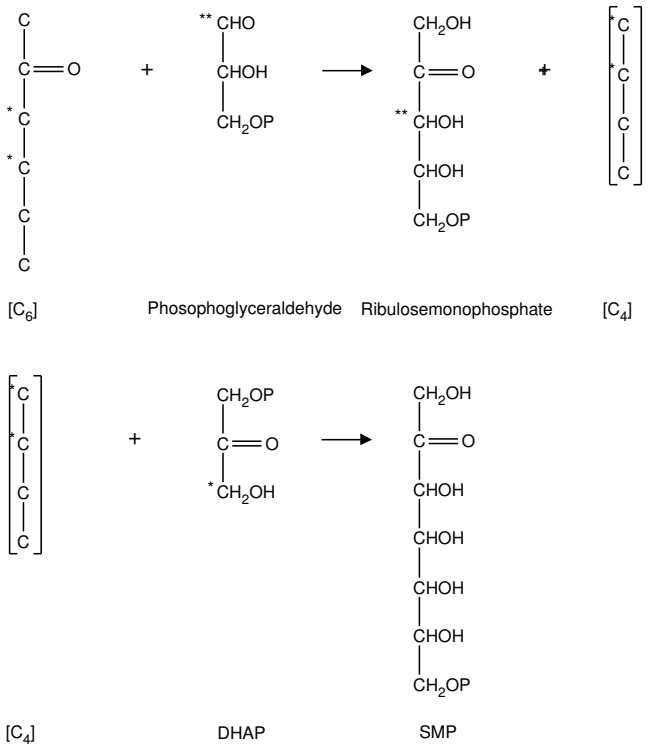

Figure V.18: A possible pathway for the formation of a C_4 fragment, which in a subsequent reaction would yield, in combination with triose phosphate, sedoheptulose monophosphate. After Bassham et al. (1954), p. 1768.

regenerate two pentoses. In the net reaction, three molecules of carbon dioxide would be reduced to one triose molecule.

The biochemical steps are spelled out in more detail in figure V.20. The primary carbon dioxide acceptor in the cycle was a ribulose molecule [C_5], presumably ribulose diphosphate. Upon its reaction with carbon dioxide, two molecules of PGA[C_3] should almost immediately be formed, which were transformed into triose or triose phosphate molecules [C_3], that is, phosphoglyceraldehyde in equilibrium with dihydroxyacetone phosphate. These were the central compounds of the cycle. This step required the input of reducing equivalents from the photochemical reactions. The triose phosphates would be the starting point for the formation of the hexose phosphates [C_6], the primary end products of photosynthesis. At the same time, they were also the starting point for the regeneration of the ribulose diphosphate as the acceptor of the next carbon dioxide. This occurred, Bassham et al. suggested, on two parallel paths. Triose phosphate, presumably phosphoglyceraldehyde, might react with glucose, upon which (through an unstable intermediate) two molecules would be released: an unknown [C_4] fragment and one molecule of

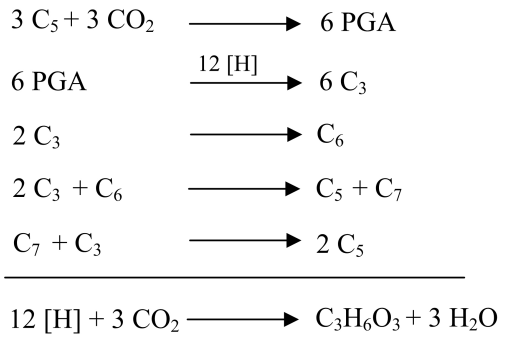

Figure V.19: The sequence of biochemical steps in the cycle in relation to the sugars. After Bassham et al. (1954), p. 1768.

ribulose monophosphate [C_5]. The [C_4] fragment would, in turn, react with another triose phosphate, presumably dihydroxyacetone phosphate, to form sedoheptulose monophosphate [C_7]. The latter would, in turn, also react with one molecule of triose phosphate, presumably phosphoglyceraldehyde, and (through an unstable intermediate) give rise to one molecule of ribulose monophosphate [C_5] and another of ribose monophosphate [C_5], both of which were readily converted into ribulose diphosphate. Upon which the cycle could start again. The cycle was repeated in the paper by Wilson and Calvin in 1955, and complemented by yet another simplified schema (see fig. V.21). As in the schema of 1954, only the central components were mentioned. This contrasts starkly to the actual development of the knowledge concerning this cycle, which greatly increased through the addition of one newly identified intermediate after the other. Once the sequence had been more or less definitively established to a sufficient degree of certainty, it became possible to deliberately simplify the cycle in order to make it as comprehensible as possible.

With this paper, the explanation of the dark reactions of photosynthesis had been reached. All the core problems were solved: integrating the mysterious five-carbon and seven-carbon sugars; finding the carbon dioxide acceptor; and establishing a full sequence of reaction steps that was in agreement with all the relevant empirical evidence. This model, with minor modifications and expansions, is still part of the commonly accepted knowledge in biochemistry today.

3.6 The Thioctic Acid Theory

By 1954, when the big question seemed to have been answered, Calvin had already turned to something new – the quantum conversion process in photosynthesis. Calvin's pet theory at the time, on which he worked intensely for more than two years (and with him most of the other people in the laboratory), was the role of thioctic acid in this process. In 2002, Benson recalled how this theory felt like an exciting intellectual adventure, "a superb concatenation of information, ideas, and experimental evidence [that] appeared to fit with all we knew of photochemical energy conversion in the chloroplast".[134]

[134] Benson (2002*a*), p. 44. See also Benson (1995).

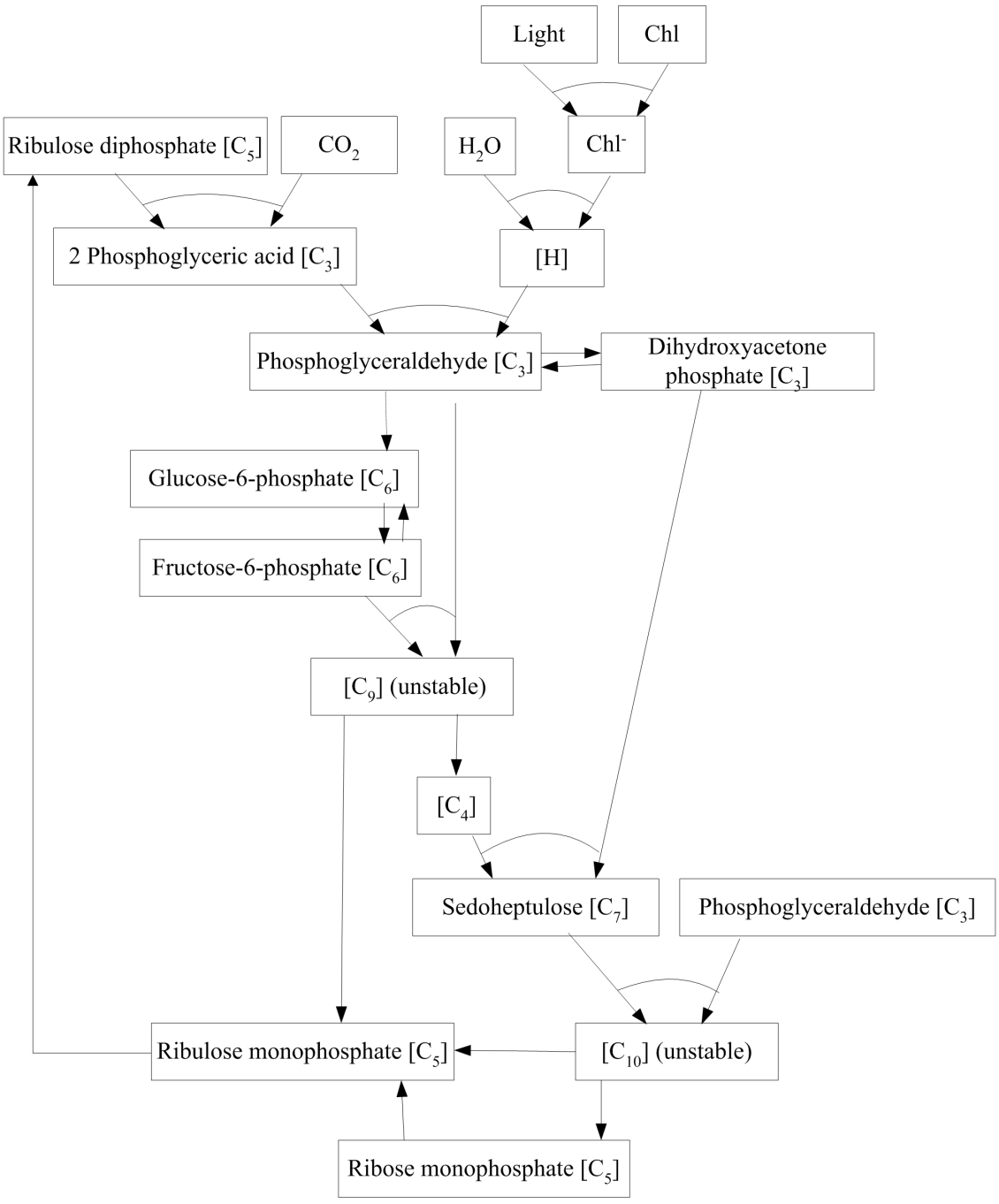

Figure V.20: The cyclic model of 1954. Reconstructed from Bassham et al. (1954).

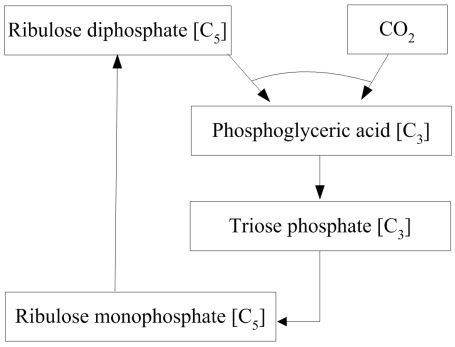

Figure V.21: A much simplified version of the 1955 cyclic model in which only the "central" compounds have been noted After Wilson and Calvin (1955), p. 5954.

Thioctic acid, a five-atom ring disulphide, had caught Calvin's attention when it was discovered to be a co-factor in biological redox reactions (today it is also known as alpha-lipoic acid or vitamin N). Calvin suspected that this was the key compound that initiated the separation of oxidising and reducing power in the first photochemical steps of photosynthesis.[135] He was able to obtain a small sample of this substance and found that the absorption spectrum, which stretched into the visible range, diverged largely from the spectrum of ordinary disulphides, which was mainly in the ultraviolet range. Calvin thought that:

> ... the excited state of the thioctic acid would react with water in a hydrolytic cleavage, giving an SH group on one sulfur atom and an SOH (sulfenic group) on the other. Thus, one of the sulfur atoms would become a reducing agent and the other an oxidant, achieving the principal result of the primary quantum conversion in photosynthesis.[136]

Calvin presented this idea so enthusiastically at one of the meetings of the American Association for the Advancement of Science that Cornelis B. van Niel "jumped up from his seat in [the] front row and, with tears in his eyes, congratulated Melvin for making the ultimate discovery of the mechanism of photosynthesis".[137] Yet, however ingenious the idea may have looked and however meticulously the evidence had been put together in a series of publications, in the end the whole model was silently dropped and never revived again. Its fate very much resembled that of the formaldehyde model of photosynthesis: even the most sensitive bioassays were unable to demonstrate the compound's presence in photosynthetic tissue; and while it was also impossible in this case to demonstrate the irrelevance of thioctic acid, the model fell into oblivion when more promising new alternatives arose (see Chapter VI). After several years of study, Calvin gave up his thioctic acid project. Remarkably, however, he did not seem to have been too despondent about the outcome. "One can survive a failed effort; even one which had involved many

[135] Cf. Calvin (1992), p. 70.
[136] Calvin (1992), p. 71.
[137] Seaborg & Benson (1997), p. 71.

man-years of work and excitement" was the lesson that Benson and others in the laboratory learned from this episode.[138]

3.7 The Fraction 1 Protein

Although Benson was also involved in the thioctic acid studies, from 1953 his main work centred round his search for the carboxylation enzyme that catalyses the first major step of the photosynthetic carbon cycle. After a series of attempts, Benson finally succeeded in preparing sufficient amounts of the enzyme's substrate, ribulose diphosphate: as he later recalled, this was "the world's supply of the pure compound with which we could assay enzymatic carboxylation using $^{14}CO_2$ and measuring fixed radioactivity which would be in the phosphoglycerate produced".[139] Benson worked on this problem with the laboratory's two microbiologists, Clinton Fuller and Rodney Quayle, and together they managed to set up a cell-free system in which the carboxylation was demonstrated to work. They wrote the following conclusion in their first publication on the theme:

> It is clear that the [cell-free] extracts contain an enzyme (or enzymes) capable of catalyzing the carboxylation of ribulose diphosphate, specifically, to form phosphoglyceric acid. No intermediates between these compounds have been detected by this method which would have detected as little as an amount corresponding to 5% of the phosphoglyceric acid formed."[140]

The next obvious step was to identify and possibly isolate the enzyme at work. With perfect timing, a young Belgian biochemist, Jacques Mayaudon, joined the Berkeley laboratory early in 1954. Endowed with a stipend for six months, he eventually stayed for a full year. Although he was originally involved in a project on bacterial photosynthesis, which had been assigned to him by Calvin, Mayaudon preferred collaborating with Benson in the latter's search for the key enzyme in the cyclic path of carbon. Both Mayaudon and Benson remembered this time as a period of feverish work; indeed, Mayaudon had no memories of doing anything other than work. He was still required to work in the bacterial project, which he carried out during the day, but at night Mayaudon worked with Benson on extracts of New Zealand spinach (which, taxonomically speaking, was not spinach at all), in the hope of identifying the enzyme of enzymes.[141] (Mayaudon, incidentally, left a rather unfavourable impression on Calvin: in addition to his peculiar preference for working with higher plants instead of algae – which was incomprehensible from a chemist's point of view – Mayaudon somehow managed to insert a rotor into the centrifuge without centering it, which completely ruined the highly valuable instrument. Thereafter, Mayaudon no longer had to work on the bacterial project.)[142] Eventually, Benson and Mayaudon succeeded in their

[138] Benson (2002*a*), p. 45.

[139] Benson (2002*a*), p. 45.

[140] Quayle, Fuller, Benson & Calvin (1954), p. 3611. On Quayle, see Kornberg (2006); see also the autobiographical account of Fuller (1999).

[141] See Moses & Moses (2000), interviews with Mayaudon and Benson. See also Benson (2002*a*).

[142] See Moses & Moses (2000), interview with Ning Pon, another member of the laboratory, to whom Calvin handed over the young charges.

task, and had crystals of the enzyme in their hands: ribulose carboxydismutase, as they had agreed to call it.[143]

This was exciting enough. However, things became even more unsettling when it began to dawn on Benson that the enzyme's activity was prevalent in an ammonium sulphate precipitate of the extract, which at the time was already well-known in the literature as the "fraction 1 protein". Isolating this protein was the achievement of the plant physiologist Samuel Wildman, who had started working on it while employed in the laboratory of James Bonner at Caltech. Since Benson frequently visited Caltech, he was familiar with Wildman and his work.

Wildman had been Bonner's postdoctoral student in the years 1944 to 1950.[144] After the Second World War, Wildman and Bonner had set out to work on plant enzymes, the activity of which they believed might be affected by the influence of plant growth hormones (which had been Bonner's focus of interest earlier in his career). From the work done by the British biochemist Albert C. Chibnall in the 1930s, it was known that the proteins of the leaves of green plants could be separated into two large classes: green insoluble proteins and non-green soluble proteins. Following this division, Bonner and Wildman decided to focus on the soluble ones. The techniques for studying proteins, however, were rather primitive; and the best thing one could do was prepare a precipitate and try to obtain the protein in question by this means. Wildman thus started to add a saturated solution of ammonium sulphate to the extracts, until a precipitate that could be collected appeared. Then more ammonium sulphate was added to the supernate, until the next portion of precipitate salted out. At about 35 per cent saturation, Wildman and Bonner found a voluminous precipitate that they called "fraction 1", in order to differentiate it from what remained in the supernate (which was called "fraction 2" and could be collected by making a concentrate of the extract through evaporation).

Wildman was able to investigate this protein on one of the first moving-boundary electrophoresis instruments, the so-called Tiselius apparatus, which had just been constructed at Caltech in the neighbouring laboratory of Linus Pauling (who was always keen on having the most up-to-date equipment available for protein characterisation).[145] In this instrument (which, among other things, comprised an optical bench of thirty feet [9.1 m] in length), proteins contained in a buffer solution were placed in a U-tube; then an electrical current was applied, which caused the charged protein molecules to migrate. According to the differences in electric charge, the different proteins would thus separate from each other. The boundaries of the sites of protein could then be made visible by passing a special beam of light through the solution.[146] Wildman remembered his findings as follows:

[143] Ning Pon recalled that there were endless discussions about what to call the enzyme. See Moses & Moses (2000), p. 9/3. In the same vein, Benson wrote on a Christmas card to Warburg: "An enthusiastic belgian [sic], Mayaudon, and I have finally succeeded in purifying the carboxylation enzyme. Should we call it 'Photosynthase' or 'ribulose diphosphate carboxylase'? It seems to be a major leaf protein." Archive of the BBAW, NL Warburg 114. Card undated.

[144] See Wildman (1992) and Wildman (2002) for autobiographical accounts of this story.

[145] Arne Tiselius, the inventor of this apparatus, received the 1948 Nobel Prize in Chemistry for analytical work done with the help of this instrument.

[146] See Wildman (2002), p. 245.

> The result was very intriguing. Without ammonium sulfate fractionation, the cytoplasmic proteins migrated as if 70% of their content consisted of a single protein. When the ammonium sulfate cut labeled Fraction 1 was tested, it migrated as a single, electrophoretically homogeneous component. Furthermore, the minimal spreading of the boundary during electrophoresis suggested the protein to be of high molecular weight.[147]

Four years later, the protein was studied yet further with the help of the ultracentrifuge, which, in the meantime, had also appeared in Pauling's laboratory. Jon Singer, who helped Wildman analyse the fraction 1 precipitate, was determined to demonstrate the lack of reliability of electrophoresis; however, he was astonished to see that according to the centrifuge pattern a minimum of 50 per cent of the soluble spinach leaf proteins was made up of a large molecular weight component, with a weight of about 600 000 (based on a sedimentation coefficient of 18 Svedberg units). Furthermore, this very protein could be demonstrated to be present in a host of other plants. Being thus homogenous according to the most rigorous tests available at the time, the fraction 1 precipitate henceforth became known as the "fraction 1 protein".

No wonder that Benson was thrilled when he realised that his carboxydismutase precipitated along the same lines as the mysterious protein "fraction 1"; he was to remember this moment of realisation as one of the most exciting times of his life. And forty years later, Wildman could clearly remember Benson's telephone call to him, in which the latter broke the good news to him. Together with Mayaudon, Benson typed out a manuscript, in the format of a "Letter to the Editor" of the JACS, in which they described their finding and mentioned that their enzyme closely resembled Wildman's fraction 1 protein. Thereupon, to the best of Benson's recollection, the following happened:

> Being a government laboratory, it was required that publications pass through an "inhouse-review": I submitted the manuscript to Melvin Calvin. The results of our tremendous efforts could have been published in 1954, but first appeared in print late in 1957 with no mention of the fraction 1 protein. Possibly Melvin did not recognize its importance – since he was unfamiliar with and disinterested in the work of Sam Wildman at Caltech. I left the laboratory at the end of 1954 and was unable to follow the work. Jacques continued in 1955 masterfully documenting our discovery. Identification of the fraction 1 protein with the carboxydismutase protein appeared in print in 1957.[148]

While the paper written up by Benson and Mayaudon was lying somewhere on Calvin's desk, another group, the research team led by the biochemist Bernard L. Horecker at the National Institutes of Health (NIH), a US Government medical research agency, succeeded in preparing purified ribulose diphosphate carboxylase, although they did not yet identify the enzyme as being the fraction 1 protein.[149] This is an impressive testimony to the ingenuity of Benson's conclusion, which was

[147] Wildman (2002), p. 245. The results were published in Wildman & Bonner (1947).

[148] Benson (2002*a*), p. 46. The paper referred to is Mayaudon, Benson & Calvin (1957); see also Mayaudon (1957).

[149] See Horecker, Hurwitz & Weissbach (1954) and Weissbach, Horecker & Hurwitz (1956).

by no means self-evident. However, when Wildman's associates Robert Dorner and Albert Kahn learned that the enzyme in question had a sedimentation constant of 18 Svedborg units, they quickly came to the conclusion that the carboxylation enzyme and the fraction 1 protein had to be one and the same.[150] After a sequence of ever more complicated, jaw-breaking names for the enzyme had been tried out, a name that was first coined by David Eisenburg at a symposium to celebrate Wildman's seventieth birthday in 1979 caught on, which is still used today: "RuBisCo" – a kind of acronym for "Ribulose-1,5-bisphosphate carboxylase".

It is not entirely clear why Benson and Mayaudon's paper on the identity of what was then called carboxydismutase and the fraction 1 protein seemingly disappeared; neither is it clear whether (and if so, how) this was related to the fact that very soon thereafter Calvin asked Benson to leave the laboratory by the end of 1954. Benson himself has repeatedly refused to comment on this episode and Calvin likewise gave no plausible explanation, one can only speculate on the possible reasons for Benson's dismissal, which might include, on Calvin's side, extreme competitiveness leading to the resentment of colleagues' achievements, combined with high unscrupulousness when dealing with staff. Benson found a position at Penn State University (Pennsylvania), and later moved on to the Scripps Institution of Oceanography in La Jolla (California). He continued to make important contributions to science; but it must have been hard for him to come to terms with the resulting situation: only had he been dismissed from the laboratory that he had to a great extent set up himself and in which he had guided so many projects to important discoveries; also the greatest reward for having discovered the path of carbon in photosynthesis, the Nobel Prize in Chemistry of the year 1961, was awarded to Calvin alone. Not only Benson, but also Kamen should, by rights, have shared the honour with Calvin. (As the Nobel Prize is not awarded posthumously, Ruben was no longer eligible for this honour.) The most extraordinary indication of the complete disregard in which Calvin held Benson, however, must be the former's autobiography, published in 1989, in which Benson received not a single mention. Fuller has very pointedly summarised the disconcerting feeling with which one is left after having read Calvin's book:

> I would like at this point to express a personal note that represents my own feelings and the recollections of many of the scientists who with me experienced the research years at the ORL in Berkeley on photosynthesis. Calvin's autobiography, *Following the Trail of Light*, represents an extremely singular view of the research carried on in the laboratory particularly in the area of the path of carbon for which he received the Nobel Prize. In all the 175 pages of his autobiography there is not one sign of Andy Benson or a mention of him. There is not one picture of Andy in a book that contains 51 photographs ranging from graduate students to the King of Sweden. There is not the citation of a single paper with Benson as an author or co-author in an extensive bibliography of over 150 references. Benson's name appears nowhere in the text and consequently is absent in the 12-page index.
>
> This appears to be an undeserved slight to a great scientist both personally and professionally who had contributed in a major way to all of Calvin's research and technology in the field of photosynthesis. Andy was a real leader

[150] See Dorner, Kahn & Wildman (1957).

> in the laboratory both intellectually and experimentally. He should have been a partner in the Nobel Prize. Al Bassham's contributions are also understated, although he is pictured and cited through the text. I know that all of us who were colleagues at Berkeley agree that it was Andy and Al who contributed greatly to our own success in future endeavors. I have no idea what may have caused this unfortunate event, but I think that history should record that the contribution of Andy Benson is not properly recognized in Calvin's autobiography.[151]

4 Concluding Remarks

Methods, Means and Goals

On the whole, though, Calvin's Bio-Organic Chemistry Group at Berkeley and the photosynthesis division headed by Benson in particular, were hugely successful. The question one should then pose is why this was the case. One of the answers most frequently given is that the methods used by the team were first rate. First, the Berkeley team had the advantage of being one of the very few research groups at the time, in terms of the team's body of knowledge and its infrastructure, that could work with carbon-14. And, second, the group was extremely lucky to come across the technique of paper chromatography for identifying the carbon compounds.

This is undoubtedly true, yet if one stopped at this point and did not look into the question any further, one would be ignoring the fact that methods neither have a self-sustained existence, mysteriously coming to people without their knowledge, nor are they usually tailor-made for any one concrete research project. Although having carbon-14 at one's disposal was a coup in itself, it still had to be administered to the plants. Knowing about the technique of paper chromatography was advantageous, but one still had to have the far-sighted intelligence to recognise its potential, as well as the skills to adapt it to the compounds under study. This was not a case of a research group deliberately picking the best-equipped tool-kit out of a range of options on offer. The Berkeley research group was highly receptive to new techniques and new instruments, highly imaginative in recognising what might be useful, and highly skilled in developing, elaborating on and adapting the available prototypes to the purposes at hand. There was also a productive division of skills and labour between the department heads: if Calvin was the person who was eager to try out the latest methods and gadgets, Benson was the one who creatively adapted them to the requirements of the laboratory.

Thus, the importance of the role of new methods, which is frequently stressed in analyses of the path-of-carbon discovery, is only one half of the story. Methods are important, and they can open up completely new ways of tackling a problem. Yet every method is only as valuable as the way it is used. Methods are the means of reaching a goal; and while a goal might not be reached if the means are lacking,

[151] Fuller (1999), pp. 9–10. See Calvin (1992) for the autobiography referred to above. The fact that autobiographical accounts can create a new reality, at least for the person who wrote it, is well demonstrated by Moses's interview with Calvin: in line with his book, but surely not in line with the facts, Calvin was unable to remember any crucial role that Benson played in the photosynthesis project: "I guess he was there [when the project was being set up], but he wasn't crucial to the action. He was there, but I don't remember him doing something that made a difference." Moses & Moses (2000), interview with Calvin, p. 1/30.

the means become meaningless if there is no goal and no actor to make them work. Take as an example Ernest Lawrence and his radioactively labelled isotopes. He had the means but was unable to put them to work to reach a non-physical goal. Ernest Lawrence thus turned first to his brother John, whom he invited to use the isotopes for medical purposes, and then to Kamen, who had the knowledge to produce and handle the isotopes. Kamen, in this case, was purely a "means" of reaching the goal.

However, Kamen and Ruben then sat down to think about what else they might be able to discover – besides a cancer therapy – using these methods. Because of their methodical competencies they came up with the metabolism experiments and then with the goal of elucidating the biochemical steps of photosynthesis; but neither goal was selected on account of the method itself. Both goals had still not been reached by the time these two chemists stopped working on the project, and Lawrence appointed Calvin to replace them. To some extent, Calvin was (from Lawrence's perspective) part of the "means" of reaching the goal. Benson was hired by Calvin following a similar rationale, with the additional advantage that Benson was a skilful experimenter and was already knowledgeable about the subject, so that he ideally complemented Calvin's own qualities. The other members of the group were not always as purposefully selected, yet it was entirely clear that once they were "on board", all their activities somehow had to contribute to the common goal. This brings me to consider in slightly more depth the kind of collaborative work that operated within the Calvin–Benson group.

Collaboration

The great advantages that the Berkeley group had, in addition to its methods, were its excellent infrastructure (thanks to almost unlimited financial resources), the highly interdisciplinary composition of the staff and the unique spirit of collaboration that prevailed in the laboratory. Calvin was certainly an exceptional thinker, but perhaps his greatest achievement at Berkeley, together with the other senior scientists in the group, lay in his ability to create an atmosphere that was characterised by a keen determination on the part of all the team members to reach a common goal through working collaboratively. "They were tied together by a desire to solve this problem, this big, hot problem, in just one little group," the biochemist Nathan E. Tolbert recalled.[152] The laboratory's inspiring and congenial working atmosphere was easily able to compensate for the fact that the Berkeley group members, for the most part, had to work with highly unpleasant and dangerous substances, notably radioactively contaminated material and highly toxic organic solvents, such as phenol. Tolbert and many others are recorded as having said that they never again experienced such a strong sense of group identity and such collective resolve.

While Calvin was, to some extent, the overarching spirit of the group, who set the general agenda, a surprising amount of the collaborative work carried out within the large group was organised by the team members themselves. As group members discussed the latest results and hypotheses, new sub-projects emerged, and although these new projects were presumably submitted to Benson or Calvin

[152]Moses & Moses (2000), interview with N. Tolbert, p. 29/20.

for approval, they were still initiatives that came about through a bottom-up approach. According to all reports, these initiatives were strongly encouraged, even if new equipment had to be installed in order to realise the ideas and new methods needed to be learned. Cost was never a limiting factor, and neither was lack of experience. In addition to these sub-projects, other types of collaborative work, on very different levels, were undertaken, ranging from the direct practical support that members gave to each other's work (for example, experiments often required more than one pair of hands) to an indirect form of collaboration (for example, the results of individual research projects were often highly significant to other members' projects). Most of the time, every person working in the laboratory was involved in more than one major project; some projects were carried out alone, others involved working cooperatively.

Of course, this way of working did cause friction at times. One of the most obvious areas of difficulty in such highly collaborative arrangements is the question of whom gets credited for which particular part of the work, which often comes down to whom is named as the first author of a publication. In the early years of the laboratory's operations, everybody involved in the work was listed as an author in alphabetical order, irrespective of their input. This, of course, favoured Bassham, Benson and Calvin, while others were always placed last, so that the custom was changed over the course of time. According to reports, authorship was later determined by Calvin on a case-by-case basis, and it was mostly felt that Calvin was usually generous in attributing credit to those who had done most of the work. Calvin co-authored most of the central papers, and there were few instances in which this was remembered by others as being inappropriate. In this respect, too, the Berkeley group seems to have tried to minimise jealousy and malevolence.

Having said this, it is also clear that Calvin's style as director of the whole project did not please everyone. Rodney Park, for example, felt that there was a distinct lack of individual freedom in the laboratory, which he believed explained why the really excellent minds did not stay there for very long; most of the donkey work was done by postdoctoral students, who spent two or three years in the Berkeley group before moving on to other places.[153] Benson thought that Calvin tended to exploit visitors and other staff for his own purposes: "Melvin was so darn clearly oriented in which way he wanted to go and he refused to let anybody distract him with their own ideas; he only wanted to push them for what he could glean that would help his ideas, which is sort of disgusting, but in other ways it was very successful."[154] A more charitable interpretation of the situation would be that this was a perfectly natural way of co-ordinating a large collective: there were a few central figures – in this case Calvin, Benson and Bassham – plus a large circle of temporary or even occasional contributors, who made a contribution and then moved on to other shores.

Less inevitable was the clear hierarchy that prevailed between the leading actors. Calvin left no one in any doubt that he was the director, although, at the same time, it was obvious that Benson also played a central part in the project;

[153] Moses & Moses (2000), interview with Park, p. 25/11.
[154] Moses & Moses (2000), interview with Benson, pp. 12/29–30.

the ensuing conflict that arose between Calvin and Benson was eventually resolved most unpleasantly. Quayle remembered the complementary roles of Calvin and Benson as follows:

> He [Calvin] would come tearing into the lab with this new idea, which you'd have to stop and listen to and he'd pull those finger joints [...] clickery click. If he felt you weren't quite, you know, keeping up with him he would sort of look at you and click, click, click. It was most off-putting. And then he'd bubble forth: it was this compound, that compound, and "You understand, Rod, do you, you're following me?" And then he would go away and, Andy, who would have listened to all this, said "Oh, that's his latest theory, is it? Well it's nonsense, it won't work because of this or that."
>
> So there was, between Andy and Calvin, there was a sort of tension, in a way. It probably was a creative tension, I think, but Andy could see reasons why something wouldn't work and he would know very well that in two days' time there would be another rush of ideas that would come in. Andy was a very good practical person, you know. He knew how the whole lab worked, what you could do with radioactivity and how to cope with it. [...] It was something that I felt was the working arrangement between the brilliant chap at the top and somebody who had seen it all happen before, perhaps sometimes a bit frustrated that perhaps not enough credit was coming his way, I don't know. There was a tension there.[155]

Calvin clearly needed Benson, not only as the co-ordinating head of the photosynthesis division but also as the central sparring partner in the laboratory. Moses recalled that the group, and in particular Benson, acted as a corrective to Calvin's over-abundant creativity: "He had plenty of critics because everybody was so intimately involved that if Calvin came up with an idea, which was somehow related to photosynthesis, there were half a dozen other people who were also very clued in on it, who could immediately respond and criticise."[156] However, after the publication of the important twenty-first paper, this arrangement no longer seemed to work and was abruptly brought to an end.

It was not only the specific relationship between Calvin and Benson that broke down. After 1956, although the project continued, the unique atmosphere had gone. When Moses asked the group members about this shift, most of them attributed it to the move from the ORL building (which was pulled down in 1959) to the new, fancy, circular building designed by Calvin. The structure was intended to encourage communication across the laboratory benches, as it had developed spontaneously in the ORL; however, most people agreed that it was not the same. This is how Bassham put it: "It wasn't ever as exciting a time as [it] was during the mapping of the path of carbon in photosynthesis. That kind of excitement left. That's not to say, though, that some of Melvin's ideas didn't stimulate other excitements in the laboratory, but it wasn't the same thing as it had been in [the] ORL."[157] What may have been the reason was that the group was no longer focused on one common goal – finding the solution to a major problem with which

[155] Moses & Moses (2000), interview with Quayle p. 3/4.
[156] Moses & Moses (2000), Moses in the interview with N. Tolbert, p. 29/23.
[157] Moses & Moses (2000), interview with Bassham, p. 7/17.

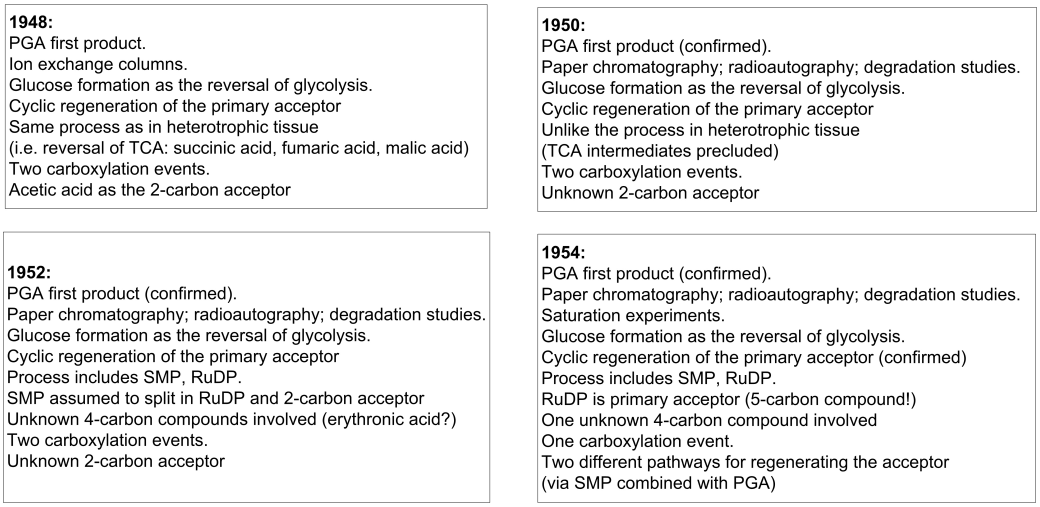

Figure V.22: Synopsis of the Berkeley group's sequence of models.

mankind had struggled for the past one hundred years, at the very least. As soon as this goal disappeared, so too did the group's identity and personal coherence.[158]

Models and Heuristics

In this section, I shall analyse the sequence of the four cyclic models that were proposed in the years 1948 to 1954 in more detail. If one reduces the models to their essential assumptions, a number of constant parameters emerge that had been assumed from the start (see fig. V.22). Among these were the assumptions that PGA was the first product of photosynthesis, that glucose formation is glycolysis run in reverse and that a cyclic regeneration of the primary carbon dioxide acceptor takes place. In terms of methods, the greatest change came around 1948, that is, when the group abandoned ion exchange columns in favour of paper chromatography and radioautography. The actual reconstruction of the cycle, of course, changed dramatically: at the beginning was the standard hypothesis that the fixation of carbon dioxide took the same path in photosynthetic and heterotrophic tissues; it followed the assumption that it was a modified version of the standard path that involved oxaloacetic acid; and in the end, there was the unexpected solution that included the newly identified substances, sedoheptulose monophosphate and ribulose diphosphate.

These were, of course, only the pathway's most important steps. Besides the major proposals for the photosynthesis model, there were many other suggestions that appeared in a number of publications and contributions; indeed, some contemporary scientists felt that far more papers were published than were justified. Not everybody was impressed by the way that science was practised in the Berkeley laboratory. In a letter of 26 December 1955, Robert Emerson told Robin Hill about the second Gatlinburg conference on photosynthesis; and although he mentioned

[158] An additional (if speculative) reason, brought forward by Govindjee, may be that the new place lacked Benson's personality. (personal communication, Govindjee, 27 April 2009).

that everyone had told him that Calvin had changed a good deal "and is earning more respect than formerly", Emerson was still rather displeased by the conduct of the representatives of the Calvin group, Bassham and Kazuo Shibata, at the conference: "They seemed to reflect the hastiness and over-confidence in very small numbers of experiments, which some of us have come to associate with Calvin's group."[159] However, Emerson had to admit that the group had made enormous advances. The biochemist Arthur Schade mentioned in a letter to Warburg that there was generally some doubt as to how many more "first products of photosynthesis" could be expected to emerge from Calvin's laboratory;[160] other actors of the time also remembered that they had observed, with some amusement, how a new intermediate of the carbon pathway would appear in the Berkeley group's publications, first with a question mark added, then without a question mark, and soon afterwards the compound would be silently dropped from any further published accounts.[161] Calvin was never afraid of making errors, not even in publications; and while there was a lesson to be learned from this attitude, as Benson mentioned (that one is able to survive even the most painful public failures), some of Calvin's colleagues felt that this characteristic tended to discredit the group's papers, or, at the very least, made their audience extremely alert to the content.

The underlying charge, methodically speaking, was that causal relationships were being postulated, although the inferences were not based on sufficiently conclusive evidence. From the collective's perspective it is not entirely clear whether this is a good or bad thing. On the one hand, this type of behaviour increases the possibilities of red herrings, on which other groups might lose their time and energy. On the other hand, it does bring themes into the public domain: the latest hypotheses were shared with others, which could be very productive and which was, at least by some of Calvin's colleagues, greatly appreciated. The biochemist Osmund Holm-Hansen, for example, underlined that, even in those cases where he was wrong, Calvin did not usually make stupid errors: "They were errors which were based on the best thinking of the time on the data available. And there's no harm in that. In fact, it's a good way of science progressing."[162] Being on top of the thinking of the time and of the available data, and not only in chemistry but in a broad range of disciplines, was, after all, one of Calvin's most extraordinary qualities. And he also encouraged this way of thinking among the members of the group, if only through their daily contact with one another. The transfer of causal knowledge from other areas of science to photosynthesis studies was crucial for successfully modelling the path of carbon in photosynthesis. In order to get to terms with this complex question, one simply could not afford to ignore potentially useful leads – even if this meant that sometimes one missed the target.

The obvious thing to do was to start with the standard hypothesis; in the first amendment, then, succinic, fumaric and malic acids were dropped as possible intermediates, since their role in the cycle had become dubious. They were replaced

[159] Cambridge University Library, Ms. Add. 9267/J.54. Emerson to Hill, 26 Dec., 1955.
[160] Archive of the BBAW, NL Warburg 819. Schade to Warburg, Nov. 1, 1957.
[161] See Morton (2007), p. 37.
[162] Moses & Moses (2000), interview with Holm-Hansen, p. 11/20.

by oxaloacetic acid: a four-carbon compound, which in a well-known reaction series was connected to all the acids that had to be eliminated via reactions known from the tricarboxylic acid cycle. This was the smallest possible change to be introduced, which at the same time explained why succinic, fumaric and malic acids were possibly present in the solution, namely as derivatives of the oxaloacetic acid. Thus, even the model variant of 1950 did not completely discard the close analogy between respiration and photosynthesis. The data still seemed to support the dominance of three-carbon and four-carbon compounds, as well as the existence of two different carboxylation reactions.

Drastic changes were required when two completely new compounds had to be incorporated: ribulose diphosphate and sedoheptulose monophosphate. Crucial data came from the degradation studies, but even before these became definitely available (in 1952) a first hypothesis was formulated that included the compounds in a way that seemed reconcilable with what was known about the reactions that similar compounds were able to undergo. It was only when the data of two different sources were accumulated that the final solution emerged. First came the fact that the straightforward hypothesis was unable to explain the curious labelling patterns of the compounds. However, this was not in itself fatal. Calvin surely would have been able to find a number of ways out of this complication. Second, however, there were the data from the saturation experiments, which indicated that ribulose diphosphate operated as the primary acceptor. This demonstration of a direct causal relevance – for the fact that the combination of carbon dioxide and ribulose diphosphate yielded PGA, while PGA, in turn, seemed directly relevant to the formation of ribulose diphosphate – resulted in the hypothesis that two carboxylation events were involved being abandoned

The decisive turning point was the recognition that ribulose diphosphate was formed on two different pathways. And although this might appear as simply piecing together a jigsaw puzzle, it is significant that the group only came up with the idea of combining sedoheptulose monophosphate with triose phosphate after evidence for the reverse reaction had been found by other research groups, for example, the team working around the biochemist Bernard L. Horecker at the NIH.[163] This recognition, combined with the knowledge that many enzymatic reactions were capable of running in both directions, fully justified, in the eyes of the Berkeley team, this unusual formation path. Note that, by contrast, the possibility of forming ribulose diphosphate by combining a three-carbon compound with a two-carbon compound although mentioned was not yet considered particularly promising.[164] The problem here was not that no potential source of the two-carbon compound was available – a path was explicitly offered, namely the splitting of a hexose into three, which would yield two-carbon compounds with the necessary labelling. However, the authors immediately objected to this alternative: "To our knowledge there exists no precedent as yet for this type of reaction."[165] In contrast to the aforementioned path (the temporary formation of a ten-carbon compound),

[163]The following references were cited in Bassham et al. (1954): Axelrod, Bandurski, Greiner & Jang (1953), Horecker & Smyrniotis (1952), Horecker & Smyrniotis (1953) and Racker, de la Haba & Leder (1953).

[164]See Bassham et al. (1954), p. 1767.

[165]See Bassham et al. (1954), p. 1767.

no causal knowledge was available that would have justified favouring this second option over the alternative.

Many red herrings were, of course, also followed up in the laboratory. In fact, the composition and cooperative nature of the group allowed Calvin and Benson to follow up almost every lead, many of which turned out to be blind alleys. A great many intermediate model hypotheses were formulated, most of which never made their way onto paper. As Kay confirmed in her interview: "Yeah, schemes always came up and were abandoned."[166] At any one time, a whole set of model alternatives were being circulated and heatedly debated, and the papers that were published represented only the tip of the iceberg. This discussion within the group was all the more necessary and useful as the Berkeley group had very soon outmatched all other laboratories in the attempt to elucidate the photosynthetic path of carbon.[167] This situation is very much in contrast with how the light reactions of photosynthesis were elucidated to which numerous actors from very different places contributed. This is the focus of the last chapter of this story, which deals with, among other themes, the discovery and elucidation of ATP production and the participation of two light reactions and two pigment systems in photosynthesis.

[166]Moses & Moses (2000), interview with Kay, p. 20/6.

[167]For some time, the University of Chicago group, which included Gaffron, Edmund Fager, Jerome Rosenberg and Allan Brown, had been competing with Berkeley, although the former team made much slower progress than their Berkeley colleagues.

Chapter VI

ELUCIDATING THE LIGHT REACTIONS (1950–61)

The 1950s are sometimes referred to as the Golden Age of photosynthesis.[1] The decade saw a dramatic increase in advances, concerning all aspects of the process, so that by the end of the period, that is, in 1961, researchers were able to propose an outline for the "light reactions" stage of photosynthesis, which is still current today: that two photoreactions involving two different pigment systems operate in series; Melvin Calvin, meanwhile, was awarded the Nobel Prize in Chemistry for having elucidated the "dark reactions" stage of photosynthesis via the Calvin–Benson–Bassham cycle.

One symptom of the dramatic changes in approach and method that occurred during this period was the fact that the technique of manometry, which had been introduced to photosynthesis research by Otto Warburg, lost its dominant position in physiological and biochemical laboratories and was superseded by the technique of spectrophotometry; it did not take long before every well-equipped laboratory was able to monitor changes in the absorption spectra of biologically significant molecules by means of a range of spectroscopic instruments.[2] These minute absorption changes – shifts in the wavelengths of the absorption maxima – frequently reflected electric fields arising from charge separations, that is, oxidations and reductions; and interestingly many of these changes were light-induced. Thus, photochemically driven redox reactions involving chlorophyll *a*, cytochromes and other compounds could be "directly" observed; and these observations became even more informative when fluorescence data were also taken into account, given the fact that fluorescence or photosynthetic utilisation are the alternative fates of excitation energy in the chlorophyll. The effects caused by the illumination of photosynthesising cells suddenly became observable at the molecular level. This provided a far more detailed basis from which to draw inferences on the biochemical and biophysical foundations of the process than all other previous techniques. Eugene Rabinowitch once said that the replacement of manometry by spectroscopy "was comparable to looking under the hood of a car in order to find out about its mechanism, as compared to studying its gas exchanges". [3] (Warburg, incidentally, never acknowledged the usefulness of spectroscopic methods.)

While investigational samples became smaller (they were reduced to the molecular or even atomic level), the laboratories in which these samples were studied were enlarged and broadened. One after another interdisciplinary centres were founded. And while most physicists working around 1950 still thought that photosynthesis was a questionable field of inquiry – too "green" a topic to be taken seriously,

[1] See, e.g., Krogmann (2000).

[2] See Clayton (2002); see also Reinhardt (2006) for a study of the advancement of spectroscopy in the chemical disciplines.

[3] Quoted in Duysens (1989), p. 68.

too strange and too complicated[4] – this attitude was soon to change. Biophysics became a respectable field of study, and although its development has usually been ascribed to the advancement of molecular genetics, some believe that this interpretation is not fully accurate.[5] Nevertheless, despite the rising popularity of photosynthesis studies, none of the research centres even remotely approached what could be described as "big science". Even in 1955, no more than about one hundred people were involved in photosynthesis research.[6]

This chapter looks at how the studies in the light reactions stage of photosynthesis undertaken in the 1950s finally culminated in the two photoreactions, two pigment systems model. This model was reached by way of a number of research paths, the results of which were surprisingly convergent. The 1960 paper by Robin Hill and Fay Bendall is best known to the public; however, its thermodynamic argument was only one aspect of the eventual model and crucially required complementary studies by others, including the two biophysicists Louis N. M. Duysens from the Netherlands and Horst T. Witt from Germany. Also noteworthy are the early contributions made by Bessel Kok, and one should not forget that Robert Emerson's finding of the Enhancement Effect in 1957 (described in Chapter IV) provided not only the incentive for many of these studies but also a convincing argument for the accuracy of the model.

The main challenge at the time was to explain how the "dark" thermochemical reactions, which had been so successfully investigated by the Calvin–Benson team at the University of California, Berkeley, in the United States (US), were related to the photochemical part of photosynthesis. More precisely, scientists need to find out how the light reactions of photosynthesis provided the necessary driving force for the dark reactions of the process (if they did so at all). It transpired, for example, that many compounds involved in the dark reactions were phosphorylated. This indicated that energy-rich phosphate bonds, which were known to be frequently provided by small molecules of adenosine triphosphate (ATP), were involved in driving these reactions. It was known that ATP was formed in the mitochondria and was the final, energy-rich product of respiration; but how this was related to the mechanism of photosynthesis, and whether or not there were alternative ways of forming ATP specific to plants, was still unknown. The second open question concerned the reducing agents required for carbon dioxide reduction. In view of the processes in respiration, photosynthesis researchers suspected that at least one of the two coenzymes di-phospho-nucleotide (DPN) and tri-phospho-nucleotide (TPN) was involved, as they were known to play a crucial role in other hydrogen-transfer reactions of metabolism.[7] But up to 1950, no one had observed any connection between these molecules and photosynthesis. How these and other

[4] Witt (1991), p. 58. citeasnounduysens89, p.65, likewise recalled that he was the first physicist working in Utrecht to take on a biophysical theme for his PhD thesis, which he completed in 1952.

[5] See Zallen (1993*b*) for a discussion of the other possible roots of "molecular" biology, among those also photosynthesis studies.

[6] This figure was taken from an estimation that can be found in Witt (1991), p. 59.

[7] The involvement of ATP and TPN or DPN was made highly probable after phosphoglyceric acid (PGA) and triose phosphate had been assumed to be the central intermediates in glucose formation: PGA can be reduced to triose phosphate with the help of pyridine nucleotides, such as TPN and DPN, only if it is first phosphorylated by ATP.

mysteries were solved, how the different parts of the jigsaw puzzle built up and were pieced together almost simultaneously in several different laboratories: these are the main threads of the narrative of this chapter.

1 The Synthesis of Reducing Power

By the 1920s, it had become clear that the reduction of an organic compound could be regarded as the acceptance of either hydrogen or electrons, while a compound's oxidation was, *vice versa*, the loss of one or the other. This was the basis for Albert J. Kluyver's theory, which cast all metabolic reactions as hydrogen transfers (see Chapter III, Section 4.1). The tendency of a compound to accept electrons from other reactants became measured in terms of "oxidation reduction potentials" or "redox potentials" for short: if a system A/A^+ had a more positive potential than a system B/B^+, this meant that, usually, A would have a strong affinity to electrons, and, hence, be in the reduced state (A), while B would preferentially donate electrons and hence become oxidised (B^+). In combinations of the two, A^+ would act as the oxidising agent, since it tends to withdraw electrons from B, while B would act as the reducing agent, since it tends to transfer electrons to A^+. The transfer of electrons between these two chemical systems would determine the redox potential, which was measured in "volts" (V) or, more aptly, "millivolts" (mV).

Carbon dioxide was known to be a very stable compound: the carbon is in its maximally oxidised state, so that a strong reducing agent (with a negative redox potential) was required in order to induce any changes. It had long been suspected that the role of reducing agent might be played by one of two "coenzymes" that were found to act in many organisms as cofactors of hydrogenases (enzymes that transfer hydrogen atoms from one compound to another). Originally, these compounds were called, rather unimaginatively, coenzyme I and II; in the course of time they were identified as being DPN and TPN. (Today they are known as nicotinamide adenine dinucleotide (NAD/NADH) or nicotinamide adenine dinucleotide phosphate (NADP/NADPH);[8] However, in order to keep the text consistent with the historical quotations, the former, outdated nomenclature has been used throughout this chapter.)

These two cofactors, DPN and TPN, also collectively termed "pyridine nucleotides", were soon found to be important agents in many of the reactions of metabolism; and it was suspected, long before it could be demonstrated, that at least one of the reactions in which they were involved played a decisive role in reducing carbon dioxide in the course of photosynthesis. The next obvious assumption was that these pyridine nucleotides were reduced in the photochemical reactions, utilising the incident light energy, and that afterwards the resultant reducing equivalents were used in the thermochemical part of the process, in which carbohydrates were formed. It was only in 1951, however, that evidence was presented that lent more support to this assumption than mere plausibility. It was also in this year that no less than three research groups (led by Wolf Vishniac and Severo Ochoa; Leonard J. Tolmach; and Daniel I. Arnon) independently and

[8] The precise abbreviation of the two coenzymes is, in actual fact, NAD^+/NADH and $NADP^+$/NADPH.

simultaneously discovered that DPN and TPN were reduced by illuminated chloroplasts: that is, the two coenzymes could be used as "Hill reagents", which are the oxidising agents in the Hill reaction (see Chapter III, Section 8.1). (The results of all three groups were published as so-called "Notes" in the journal *Nature*.)[9] This observation was surprising, since up to then only electron acceptors with redox potentials more positive than about +40mV were known to be reduced by isolated chloroplasts, while the standard redox potential of TPN/TPNH was -320mV. Thus, it transpired that chloroplasts were able to generate a much more electronegative redox potential than had previously been thought, which implied that they were able to drive many energy-requiring reactions in plants.

The first note in the series, published on 12 May 1951, was a paper jointly authored by the microbiologist Wolf Vishniac, then still a postdoctoral student at Stanford, and the biochemist Severo Ochoa of the New York University School of Medicine, who would later win the 1959 Nobel Prize in Physiology or Medicine. Vishniac and Ochoa reported that they had observed the reduction of DPN and TPN by illuminated chloroplasts. At the same time, the authors wrote, molecular oxygen was released. The experimental trick that Vishniac and Ochoa applied was to "trap" the photoreduced pyridine nucleotides (which were very unstable), by coupling the photoreduction process to the formation of a stable reaction product. This prevented any energetically favoured back reactions from occurring, since the reduced pyridine nucleotides, with their negative redox potential, tended to donate their electrons to other compounds. Vishniac and Ochoa suspected that the prevalence of these back reactions explained the failure of earlier researchers to find compounds with a relatively negative redox potential reduced by chloroplasts. Vishniac and Ochoa added a "malic" enzyme to the solution: an enzyme of the cell's cytoplasm, which catalysed the carboxylation of pyruvic acid to malic acid - a reaction that was known to be dependent on the presence of reduced DPN or TPN. The fact that this reaction ran smoothly in the illuminated chloroplasts of the experiment was taken as evidence for the light-driven reduction of at least one of the two coenzymes (see fig. VI.1).[10]

Similar results were reported four weeks later, on 9 June 1951, in a second "Note" to *Nature* written by Leonard J. Tolmach of the University of Chicago (US). Tolmach believed that not only was TPN reduced in the chloroplasts; it also catalytically promoted oxygen production under these circumstances: "Addition of small amounts resulted in yields of oxygen thirty to forty times the equivalent of the added triphosphopyridine nucleotide," he wrote. The same was observed when DPN was added, and, as Vishniac and Ochoa did, Tolmach agreed "that pyridine nucleotides might be reduced photochemically by illuminated chloroplasts and that these reduced co-enzymes could be utilized by enzyme systems for reductive fixation of carbon dioxide".[11] This was again confirmed in a third "Note" to *Nature* on this topic submitted by Daniel I. Arnon of the University of California, Berkeley

[9] Vishniac & Ochoa (1951), Tolmach (1951*a*) and Arnon (1951).

[10] Vishniac & Ochoa (1951).

[11] Both quotes: Tolmach (1951*a*), p. 947. For the idea that TPN or DPN might be used in carbon dioxide reduction, Tolmach referred to Ochoa, Veiga Salles & Ortiz (1950) and Ochoa (1950).

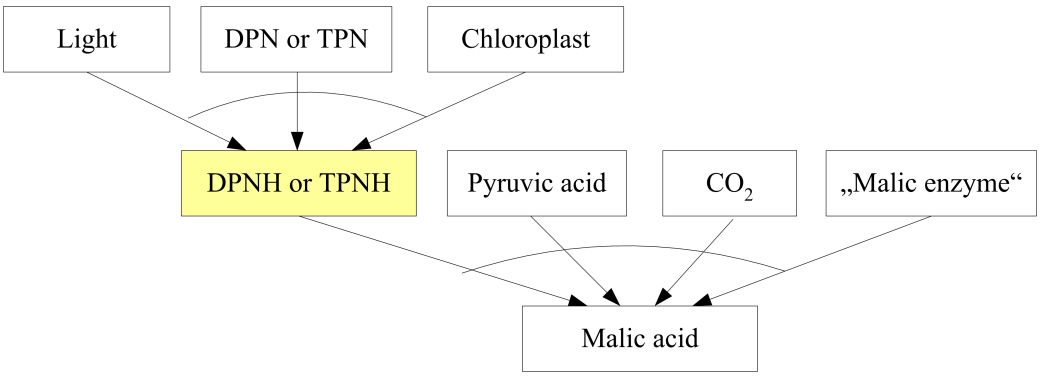

Figure VI.1: The process underlying the experiment undertaken by Vishniac & Ochoa (1951): the production of malic acid by the "malic enzyme reaction" is dependent on the presence of reduced DPN or TPN, from which it was inferred that they were produced in illuminated chloroplasts.

(US), and published on 23 June 1951; in addition, Arnon had found that TPN was more easily reduced by isolated chloroplasts than DPN.[12]

After this series of mutually reinforcing papers, there could no longer be much doubt that chloroplasts were capable of photoreducing pyridine nucleotides and thereby of developing a negative redox potential of more than -320mV. Controversial opinions prevailed, however, as to the implications of this observation for the mechanism of natural photosynthesis in living cells. The hope was, of course, that chloroplasts were also able to use pyridine nucleotides as oxidants for the splitting of water in plants; and that green plants would thus be able, through appropriate (although still unknown) enzyme systems, to use the reduced pyridine nucleotides for the reduction of carbon dioxide to carbohydrates. This seemed all the more plausible as DPNH and TPNH could clearly be used to reduce carbon dioxide while malate was being formed (in the course of the"malic enzyme" reaction described above; see also fig. VI.1). However, one of the obvious points of criticism was the fact that, in all three laboratories, the reduction of the pyridine nucleotides was observed at rates that were about fifty times smaller than would be required for the process of photosynthesis in plants to take place.

An interesting story lies behind the publication of these three papers. At the time Tolmach was working with Hans Gaffron's group at the University of Chicago (Gaffron became Director of the Photosynthesis Laboratory after James Franck had retired in 1947); and on 2 February 1951, Gaffron sent a draft of Tolmach's note (which had just been submitted to *Nature*) to Robin Hill, requesting the latter's opinion. [13] Hill replied on 12 February: "You can imagine how delighted I am to hear that a bridge head is now established between the chloroplasts and the coenzymes" – Hill had longed to see evidence for the fact that the "Hill reaction"

[12] Arnon (1951). That TPN was reduced in preference to DPN was later confirmed, e.g. in Jagendorf (1956).

[13] Cambridge University Library, Ms. Add. 9267/J.61. Gaffron to Hill on 2 February, 1951.

was, in fact, related to photosynthesis not only in terms of oxygen release but also carbon dioxide reduction. However, despite his general delight, Hill seriously advised Gaffron to withdraw the note in its current form, and write instead a more extended paper which included the precise experimental protocol and the actual data. Without these details, Hill argued, the information given in the note was almost meaningless. This blunt but well-meant comment was welcomed by Gaffron, who in his next letter admitted that he had not been entirely happy with the paper either.[14] Tolmach quickly wrote an extended version of the paper, submitted it to the *Archives of Biochemistry* – and Hill promptly gave it a positive review.[15]

Yet, only a few days after Hill had sent off his referee's report to the editor of the *Archives of Biochemistry*, he hastened to inform Gaffron about the latest developments: Hill had been appalled to see that Vishniac and Ochoa had, in the meantime, submitted a "Note" to *Nature* that was almost identical in scope and content to the one submitted earlier by Tolmach. Clearly feeling guilty about having deprived Tolmach of being the first to publish a paper on the topic, Hill informed Gaffron that he would ensure that Tolmach's note was published as soon as possible and supplemented by at least one table of data.[16] This was much appreciated by Gaffron, who had noticed how much attention Vishniac and Ochoa's results had been receiving in the US prior to publication. Furthermore, Gaffron mentioned that "Arnon also seems to have rushed into the field".[17] The Chicago group was naturally interested in getting its share of the credit, although Gaffron still considered that Hill's original criticism was fully justified. Hill did as he had promised, and Tolmach's note appeared in *Nature* in an only slightly altered form. And despite Hills's reservations about the genre of "Notes" to *Nature*, a letter written by him at the end of April to Robert Emerson again demonstrates how enthusiastic Hill was about the general finding that DPN and TPN were reduced in chloroplast suspensions; Hill considered that this discovery finally put an end to the idea that carbon dioxide might be directly reduced through the action of light on chloroplasts:

> The new achievements of Ochoa, Tolmach & Arnon one now feels very cheerful about – the biochemical part does look encouraging. Not that it yet indicates the whole solution about CO_2 but it really is good to see this new escape from the bold "hν + CO_2" picture, even if it is to be only temporary. I think Tolmach's work gives the most interesting clues – though it needs the other two to support it, in fact each of the three has its own specific contribution.[18]

[14] Cambridge University Library, Ms. Add. 9267/J.61. Gaffron to Hill on 6 March, 1951.

[15] Cambridge University Library, Ms. Add. 9267/J.61. Hill's referee's report to the journal, dated 11 April, 1951. See Tolmach (1951*b*) for the published paper.

[16] Cambridge University Library, Ms. Add. 9267/J.61. Gaffron to Hill on 16 April, 1951.

[17] Cambridge University Library, Ms. Add. 9267/J.61. Gaffron to Hill on 21 April, 1951.

[18] Hill to Emerson, 28 April 1951, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Hill, Robin, University of Illinois Archives. By the end of April, Hill had seen all three notes (and assumed that Emerson had, too), so clearly, the notes or at least their results had been circulated in advance to being published.

Emerson, however, was far less enthusiastic in his response. He was not at all convinced that these findings helped to explain photosynthesis in plants – after all, no one had demonstrated that reduced TPN was causally relevant to the reduction of carbon dioxide. He wrote to Hill, on 8 May 1951:

> You speak optimistically about the work of Ochoa, Tolmach, etc., and I agree that it's interesting to learn more about the chemical reactions which can be brought about by illuminated chloroplasts. However, I heard yesterday that the pyruvic acid reduction previously reported by Vishniak [sic] to be carried out by reduced TPN can be just as well accomplished by illuminated chloroplasts without TPN. People here were jumping to the conclusion that the pathway of photosynthesis must be via the production of reduced TPN by illuminated chloroplasts. This may still be the case, but the experiments up to date seem to indicate only that we must add TPN (and DPN) to the growing list of substances which can be reduced by chloroplasts. I suppose it is understandable that each person finding a new reduction process hopes it will prove to be the key to the mechanism of photosynthesis. But until there is real evidence of carbon dioxide reduction, and of changes in reduction level large enough to require the energy of several quanta of red light, it seems to me that only moderate claims of progress are justifiable.[19]

This rather sober evaluation of the results was in stark contrast not only to Hill's views but also to the occasionally overly optimistic tone that the publications coming from, in particular, Arnon's laboratory at Berkeley were about to receive. In the next section, I shall examine more carefully the work done by the Berkeley group in the context of the discovery of the light-driven production of ATP, independent of respiration.

2 Photophosphorylation

2.1 Energy-Rich Phosphate Bonds and Photosynthesis

In 1929, the German chemist Karl Lohmann found that fermentation was linked to the formation of molecules of ATP, which could be stored in the cells for several hours.[20] Then, in the 1930s, the Russian physiologist Vladimir A. Engelhart and his collaborators discovered that muscle contraction required ATP, the Danish biochemist Herman Kalckar established (in 1937) that the formation of ATP was linked to cell respiration, and Otto Warburg elucidated how adenosine diphosphate (ADP) was phosphorylated to ATP during glycolysis.[21] Thus, by the end of the 1930s, the central role of ATP in the organism and its intricate linkage to energy-producing processes such as fermentation and cell oxidation were beyond any doubt. In fact, more and more metabolic redox reactions were demonstrated to be linked to the cleavage and formation of ATP. It seemed that most, if not all, higher organisms were able to store the energy yielded from exergonic processes as ATP, the splitting of which could, in turn, release this energy again in order to promote the occurrence of endergonic processes.

[19] Emerson to Hill on 8 May 1951, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Hill, Robin, University of Illinois Archives.

[20] Lohmann (1929).

[21] See, e.g., Engelhardt (1930) and Engelhardt (1939); Kalckar (1937) and Warburg & Christian (1939). See Maruyama (1991) for a historical account of the discovery of ATP and its structure.

The most influential contributions to this emerging field of phosphate metabolism studies were provided in 1941 in the form of two simultaneously and independently written reviews of the phenomenon and its bioenergetic implications, authored by the aforementioned Kalckar and his German colleague, friend and mentor Fritz Lipmann (both of whom would eventually find themselves working in the US, due to political circumstances).[22] Lipmann, who left Nazi Germany in 1932 (and would win the 1953 Nobel Prize in Physiology and Medicine), had met Kalckar 1934 in Copenhagen, in the laboratory of the Danish physiologist Ejnar Lundsgaard, where Kalckar had just started his PhD studies; this encounter was the start of a life-long friendship. When Lipmann went to Copenhagen, he was already deeply interested in the biological functions of phosphorylation reactions, and not only pursued these questions in his own research but also closely followed the work done by Kalckar on the mechanism of oxidative phosphorylation. It is one of the striking but not inexplicable coincidences in the history of science that Kalckar and Lipmann both embarked on a seminal review of the "phosphate problem" at the same time, that is, around 1940.

It was in Lipmann's 1941 paper that the notion of "energy-rich phosphate bonds" was explicitly introduced, symbolised by the famous "squiggle" that is usually attached to the phosphorus atom of a phosphate group (∼Ⓟ). This symbol became the accepted notation of linkages, such as the pyrophosphate bonds in ATP, the hydrolysis of which causes a relatively large energy release. The two papers by Kalckar and Lipmann were widely received, and at least among biochemists the concept quickly met with consent, if not enthusiasm (although Lipmann recalled that organic chemists *sensu stricto* and physical chemists were outraged by the proposal).[23] It eventually transpired that ATP might be the solution to the problem of how the energy gained from the decomposition of carbohydrates could be chemically preserved and transferred in a stabilised form to other, endergonic reactions of metabolism (that is, "unfavourable" reactions that did not run spontaneously).[24] In view of these developments, it was soon speculated that ATP was also the source of energy for carbon dioxide reduction in photosynthesis. In 1943 Samuel Ruben was the first to develop a general model of carbon dioxide fixation in this vein: he assumed that the carboxylation of an organic residue, which acted as primary acceptor, was coupled to the splitting of an energy-rich phosphate bond of the ATP type (see the discussion in Chapter V, section 1.3, p. 264).[25]

It has already been mentioned that even his close friend and collaborator Martin Kamen was sceptical about the value of Ruben's suggestion; however, although this attitude was held by most of their colleagues, the idea was enthusiastically picked up by a handful of other scientists, including Emerson. Together with the plant physiologist J. F. Stauffer and the microbiologist Wayne W. Umbreit, Emerson

[22] See Kalckar (1941) and Lipmann (1941). Information on Kalckar can be found, e.g., in Kennedy (1996), and on Lipmann in, e.g., Jencks & Wolfenden (2000), Kleinkauf, von Döhren & Jaenicke (1988) and Kennedy (2001). Autobiographical accounts are provided in, e.g., Lipmann (1971), Kalckar (1974) and Kalckar (1991).

[23] See Lipmann (1971), p. 37.

[24] See Fruton (1972), pp. 363ff. for a general account of how the importance of ATP in intracellular respiration emerged.

[25] See Ruben (1943) for the paper in question.

published a paper in 1944 in which they pushed Ruben's principal suggestion a little further, although the authors admitted that it was impossible at the time to argue conclusively for the accuracy of their concept, "since undoubtedly other conceptions could equally well fit the facts observed".[26] Emerson and his co-workers proposed the following:

> The function of light energy in photosynthesis is the formation of "energy-rich" phosphate bonds. According to this view, the light energy absorbed by the chlorophyll system is converted more or less immediately, into "energy-rich" phosphate bonds [...] which furnish the energy for the remainder of the photosynthetic process. [...] We therefore conceive of the entire process of photosynthesis (the fixation, reduction, and synthesis of organic molecules from carbon dioxide and, in green plants, the production of oxygen from water) as being "dark reactions" which could be accomplished without the use of light if one were able to substitute into this system the "energy-rich" phosphate compounds which actually result from the absorption of light by the chlorophyll system. [...] Therefore, the light *per se* is not essential for photosynthesis, but some result of the absorption of light is essential.[27]

Thus, while oxygen release had been dropped from the list of essential properties for the phenomenon of photosynthesis in the 1930s, as a direct consequence of the work done by Cornelis B. van Niel, now the action of light was also being seen as replaceable if the necessary compounds could be provided in another way. Again, the relevant clues came from comparing photosynthesis in plants with the metabolism of bacteria. The authors referred, in particular, to the recent elucidation of the mechanism of energy transport in *Thiobacillus thiooxidans*: an autotrophic, chemosynthetic sulphur bacterium, which gained the energy required for carbon dioxide reduction from oxidising sulphur to sulphuric acid. It was found that this bacterium could oxidise sulphur in the absence of carbon dioxide and store at least a portion of the energy in a form that could later be used for carbon dioxide fixation under conditions in which sulphur oxidation was impossible. Thus, the two partial processes - the oxidation of sulphur and the reduction of carbon dioxide – were coupled but could later be separated, much like the photochemical production of oxygen and carbon dioxide reduction in green plants. It was demonstrated that the formation of the unknown "energy storage material" was accompanied by a substantial uptake of inorganic phosphate, while during carbon dioxide fixation an inorganic phosphate release was observed. From this and other pieces of evidence, it was concluded that the energy gained from sulphur oxidation was stored as ATP molecules.

In view of these findings, Emerson, Stauffer and Umbreit asked whether photosynthetic organisms might also use energy-rich phosphate bonds in the form of ATP to move the energy gained from the light-induced splitting of water to the endergonic process of carbon dioxide reduction. In order to learn more about this possibility, the authors emphasised, they would need to integrate fully the study of phosphorylation into the study of photosynthesis. First of all they would have to determine whether or not phosphorylation was an important part of the

[26] Emerson, Stauffer & Umbreit (1944), p. 107.
[27] Emerson et al. (1944), p. 107.

metabolism of photosynthetic cells. However, Emerson et al. admitted that, at the time, it was impossible to differentiate between the products of oxidative phosphorylation, which were the result of respiratory processes, and the potential products of photosynthetic, light-induced phosphorylation. Their own experimental data suggested that phosphorylation was, indeed, taking place in photosynthesising cells (which was hardly surprising, since these cells were known to respire), while there were only a few indications that the underlying mechanism of ATP formation was not entirely the same as the mechanism that had been found in animal and bacterial cells.

Emerson, Stauffer and Umbreit's proposal, just like Ruben's earlier attempt, was not enthusiastically received by their colleagues. In the first volume of his two-volume seminal monograph, published in 1945 , Rabinowitch rather sceptically commented on the possible role of ATP in photosynthesis. The problem of photosynthesis was a problem of energy *accumulation*, Rabinowitch underlined, since so much energy was required to reduce carbon dioxide. Bearing this in mind, it seemed like a step in the wrong direction to *dissipate* the energy of red light quanta (43 kcal per einstein) by storing it in high-energy phosphate bondings of only 10 kcal per molecule (mole).[28] Rabinowitch concluded:

> To sum up: we think it unlikely that the bulk of the light energy utilized in photosynthesis (or of the oxidation energy utilized in chemosynthesis) is first converted into phosphate energy. Furthermore, if phosphorylation does play an auxiliary role in photosynthesis (e.g., in the way envisaged by Ruben) – which is by no means certain – we think it much more probable that the required high-energy phosphates are supplied by nonphotochemical oxidation processes than that light quanta are diverted for their synthesis.[29]

These objections based on energetic considerations were strengthened by the results that Samuel Aronoff and Melvin Calvin published in 1948, on their studies incorporating radioactively labelled phosphorus into ATP in isolated chloroplasts: "Using radioactive phosphorus, no direct connection between gross formation of organic phosphorus compounds and photosynthesis or photochemical reductions has been found to occur" is how they summarised their finding.[30] Thus, in view of these results, it seemed that searching for a unique mechanism of phosphorylation during photosynthesis would be a road to nowhere.

Far more promising, however, was an alternative model that was proposed by Vishniac & Ochoa (1952*b*). By the early 1950s, it had transpired (mostly thanks to the studies of the Calvin–Benson group at Berkeley) that phosphorylated compounds were prominently present in the photosynthetic reduction of carbon dioxide; thus, there had to be a way to account for their formation during photosynthesis. It was a clever (and also parsimonious) move to draw on the recently published results of Albert L. Lehninger (1951), who had demonstrated that the site of ATP production during respiration was the mitochondrion. Hence, Vishniac and Ochoa suggested that photosynthesis was accompanied by oxidative phosphorylation, which took place in the mitochondria. The necessary ATP would

[28] One einstein is defined as one mole of photons.
[29] Rabinowitch (1945), pp. 229–230.
[30] Aronoff & Calvin (1948), p. 357.

be formed in these organelles, and then they would be transported to the sites of carbon dioxide reduction.[31] Vishniac and Ochoa had found that radioactively labelled ATP could be formed in artificially reconstituted systems: illuminated suspensions that contained spinach chloroplast grana, mitochondria, DPN and phosphorus-32, all of which seemed to be essential components. The assumption was that the pyridine nucleotides were reduced by the illuminated chloroplasts (as earlier observations had indicated) and were subsequently oxidised when they were coupled to a phosphorylating system to yield energy-rich phosphate bonds, although Vishniac and Ochoa thought that this latter process did not occur in the chloroplasts but in the mitochondria. The authors formulated their conclusion as follows:

> [T]he incorporation of P^{32} into ATP is dependent on light, oxygen, and DPN. No incorporation of P^{32} takes place in the absence of particles capable of carrying out oxidative phosphorylation. It is suggested that in photosynthesis energy-rich phosphate bonds are generated by the mechanism outlined above [that is, the light-induced formation of DPN coupled to a light-independent, oxidative formation of ATP in mitochondria].[32]

This suggestion also appeared convincing from another angle: if the photochemical reduction of DPN was coupled to oxidative phosphorylation by themitochondria, this could, in addition, explain the limited success of the Hill reaction to find any traces of carbon dioxide reduction in isolated chloroplasts. According to Vishniac and Ochoa's model, this was self-evident, since in Hill-type suspensions there were no mitochondria present to provide the necessary ATP. For the next couple of years, this became the accepted standard hypothesis: chloroplasts were the sites of light absorption and water splitting, as well as of TPNH (or DPNH) formation, while ATP was assumed to be produced in the mitochondria, and the fixation of carbon dioxide presumably occurred in the cytoplasm. The latter assumption was endorsed by the finding that the "malic" enzyme was present in ample amounts in the cytoplasmic fluid of plant cells (but not in the chloroplasts themselves). Even when it slowly transpired, through the advances made by the Calvin–Benson group, that the "malic" enzyme itself had no bearing on photosynthetic carbon dioxide fixation, the finding of further carboxylases and other enzymes in the cytoplasm made the latter the most probable place in which other enzymatic reactions would occur. A reconstruction of this "compartment" model is given in figure VI.2.

2.2 The Standard Idea Challenged

This standard picture was soon to be challenged. The first decisive finding was made by the biochemist Bernard L. Strehler. While still a graduate student at the Johns Hopkins University Medical School, Baltimore, Strehler had contributed decisively to finding out what makes fireflies glow. In 1949, he had identified an enzyme, which he named "luciferin", and established that this substance gave off light when it was combined with ATP.[33] Strehler moved on to the Oak Ridge National Laboratory in Tennessee, where he met William Arnold, with whom

[31] See Vishniac & Ochoa (1952*a*), Vishniac & Ochoa (1952*b*) and Ochoa & Vishniac (1952).
[32] Vishniac & Ochoa (1952*b*), p. 502.
[33] Strehler & McElroy (1949).

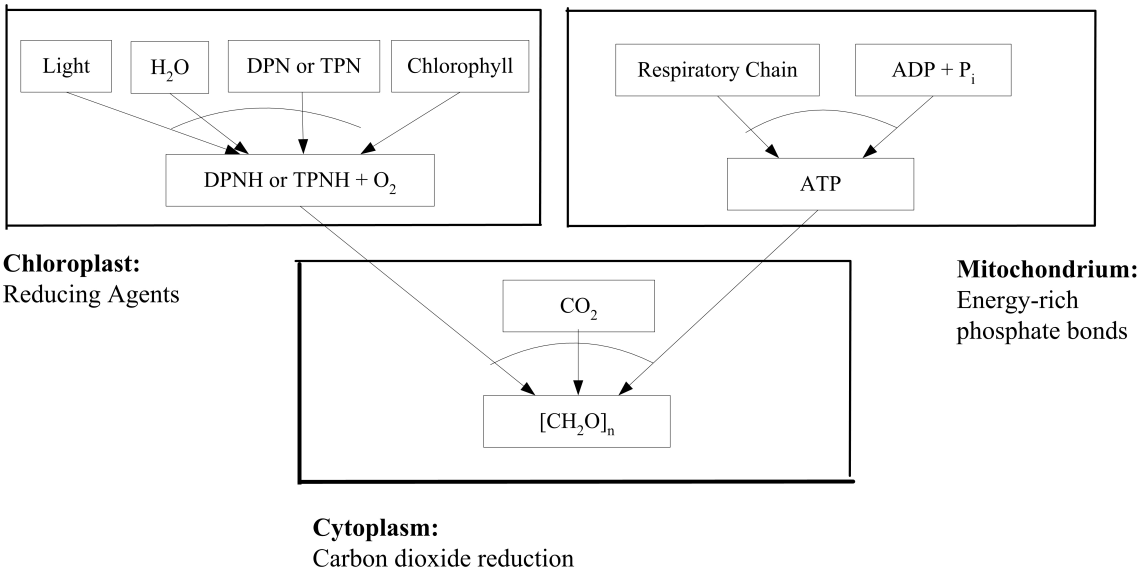

Figure VI.2: The standard model of photosynthesis according to Vishniac & Ochoa (1952b): three partial processes are identified (the production of reducing agents, the production of energy-rich phosphate bonds and the reduction of carbon dioxide), which are located in three different compartments of the cell (the chloroplast, the mitochondrion and the cytoplasm)

he began a long and fruitful collaboration.[34] Starting from Strehler's previous research, the two men wanted to find out whether ATP was formed as a result of photosynthesis; the firefly enzyme seemed to provide them with a powerful and very sensitive indicator of ATP formation. Instead, they found, first of all, that, after being illuminated, the chloroplasts gave off light even without the addition of any luciferin: Strehler and Arnold had discovered the phenomenon of delayed fluorescence in photosynthesising systems, which strongly indicated, they believed, that some of the first steps of photosynthesis were reversible.[35] However, a short while later, in 1952, Strehler established that ATP is, in fact, formed in plants immediately upon illumination, and that the site of formation is the *chloroplast*; he presented these findings at the first Gatlinburg conference on photosynthesis in 1952, where, incidentally, he was the only speaker on ATP and photosynthesis. It was clearly not yet regarded as a hot topic.[36] One year later, in 1953, Strehler presented the first detailed suggestion as to how ATP might be produced in a

[34] See Strehler (1996) for an autobiographical account.

[35] Strehler & Arnold (1951) and Strehler (1951). They only succeeded in getting the originally intended experiment to work ten years later, with the help of red filters; see Strehler & Hendley (1961).

[36] According to Strehler, both Arnon and Vishniac received a copy of Strehler's papers on ATP formation at the Gatlinburg conference; however, neither acknowledged this obvious source of inspiration in their later papers on the subject; see Strehler (1996), p. 14. See also Strehler's comment on his personal homepage, accessed in September 2009 at http://web.archive.org/web/20021207070619/fihg.org/founder1.htm .

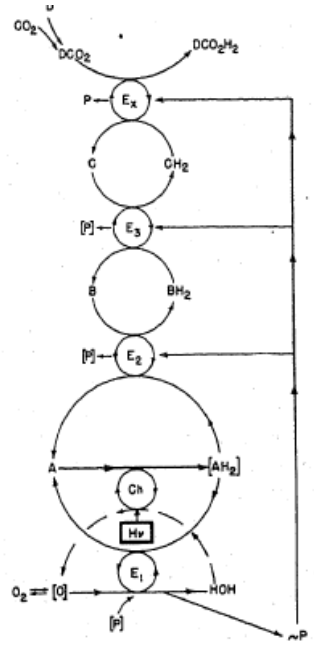

Figure VI.3: Reproduced from Strehler (1953), p. 75. Legend of the symbols: Ch = photochemical apparatus; Hν = light; AH_2, BH_2, CH_2 = intermediate reductants; [O] = photochemically produced oxidant, precursor to O_2; D = "C_2 acceptor" molecule or other acceptors; $\sim$ P = ATP; DCO_2H_H = primary fixation-reduction product; E_1 = terminal oxidase; E_2, E_3, E_x = intermediate transhydrogenases.

light-induced mechanism in plants, combined with the suggestion that a major portion of this ATP might immediately be used up again in order to produce reducing agents.[37] As one can take from one of the illustrations in Strehler's text (reproduced in fig. VI.3), the concept of step-by-step oxidation and the reduction of intermediates, with ATP formation at one of the transition points, clearly existed in 1953, even though all the details were still unknown.

These first steps towards a new concept of photosynthetic ATP formation were complemented in 1954 by the work carried out by Daniel I. Arnon's group at Berkeley. Born in Warsaw, Poland, Arnon had trained as a plant physiologist at the University of California, Berkeley and received his PhD there in 1936.[38] Arnon had been a student of Dennis R. Hoagland, who is still well-known for the pioneering work he did on plant and soil interrelations; therefore, it is not surprising that Arnon's early research focused on plant nutrition and the role of trace elements in plants. In 1941, Arnon was made an Assistant Professor at

[37] Strehler (1952) and Strehler (1953). The methods were developed in Strehler & Totter (1951).

[38] On Arnon's life and work, see, e.g. Buchanan (1995) and Buchanan (2001). See Melis & Buchanan (1995) for a special issue of *Photosynthesis Research* dedicated to Arnon.

Berkeley, and he remained at the university for the rest of his working life; in 1961 he was appointed the first Director of the Department of Cell Physiology, which developed into a centre of photosynthesis research.

In an autobiographical account, Arnon recalled how he felt increasingly uneasy with the standard hypothesis that the ATP required for photosynthesis was formed in the mitochondria. Among other things, he found this to be fundamentally at odds with well-known observations of plant cytology:

> In some of the most specialized photosynthetic tissues, such as the palisade parenchyma of leaves, chloroplasts were known to be the dominant cytoplasmic bodies. The few mitochondria present could not possibly supply the ATP needs of photosynthesis whose rate in saturating light can be 30 times higher than the rate of respiration. I decided, therefore, to continue the search for a "photosynthetic" site of ATP formation.[39]

It is highly unlikely that any of the biochemists working at the time had even considered looking at plant tissues. Yet, even without recourse to the cytological properties of plant tissues, the standard hypothesis was unable to explain how photosynthesis could be dependent on respiratory products, if the rates of photosynthesis in sunlight were so much higher than those of respiration. Arnon set out to scrutinise this problem with two close collaborators: Mary Belle Allen, who earlier in her career had worked with Charles Stacy French and van Niel, and F. Robert (Bob) Whatley, who had received his PhD while working in Hill's laboratory at the University of Cambridge (UK) in 1946.[40]

The group started off by investigating the photosynthetic capacities of the chloroplasts of green algae, the usual, convenient model organism of photosynthetic studies. However, after a series of unsuccessful attempts, Arnon's group turned to leaf tissue instead, with spinach proving to be the most appropriate source of isolated chloroplasts. Yet, in an important respect, Arnon and his collaborators deviated from standard investigative procedures: they decided not to use the membranous fraction of the chloroplasts – that is, chloroplast fragments obtained by ultracentrifugation – as Vishniac & Ochoa (1952*b*) and others had already found that these grana could not produce ATP. Instead, Arnon and his team chose to investigate *whole* chloroplasts, including their soluble content: this proved to be a fortunate decision, since it turned out later that the soluble fraction of chloroplasts contain the necessary catalysts for photophosphorylation as well as the enzymes required for reducing carbon dioxide. [41] Arnon's group developed an ingenious procedure for isolating these whole spinach chloroplasts. Among other things, they used isotonic salt solutions (NaCl) instead of the more usual sugar solutions, which had been developed in Hill's laboratory: Arnon's team wanted

[39] Arnon (1984), p. 258.

[40] The history of how the chloroplast was established as the unit of complete photosynthesis and, in particular, how photosynthetic phosphorylation was discovered at Berkeley and elsewhere, has been told many times and from different perspectives. The author gratefully acknowledges many of these accounts, which were duly consulted when writing this chapter, notably Arnon (1977), Arnon (1984), Arnon (1987), Arnon (1988), Frenkel (1993), Frenkel (1995) and Shen & Wei (1998).

[41] See Arnon (1984), p. 259.

to avoid using sugar, since it was a potential confounding factor: it was possiblly used as an additional source of energy and metabolites. If one wanted to find out the *photosynthetic* capacities of chloroplasts, light ought to be the only source of energy. Microscopic observations were employed to ensure that the structure of the chloroplasts thus gained was unimpaired; and, finally, they double-checked that the suspension was free of mitochondria, so that any support of the process by oxidative phosphorylation was precluded.[42]

The team found that not only were these intact, illuminated chloroplasts able to produce oxygen; they also accumulated ATP if supplied with the substrate of phosphorylation, adenosine monophosphate (AMP), and inorganic phosphate. When bicarbonate was then added, the system was able to reduce carbon dioxide at constant rates for about one hour. An analysis of the products yielded an insoluble compound, which was identified as starch, and several soluble compounds, which included the major components of the photosynthetic carbon reduction cycle recently proposed by the Calvin–Benson team. In their publication of 1954, Arnon's group summarised their findings: "In the light of our present evidence, isolated chloroplasts emerge as remarkably complete cytoplasmic structures, equipped to carry out not only oxygen evolution but also carbon dioxide fixation and the conversion of light into chemical energy." [43] This was the process that Arnon dubbed "photosynthetic phosphorylation", in order to distinguish it from oxidative phosphorylation, which was known to occur in the course of respiration. It was first presented at the VIII International Botanical Congress held in Paris in July 1954.[44]

Yet, the accumulated evidence for the standard hypothesis – that all the cell's ATP was produced in the mitochondria – was far too weighty to be simply swept aside. In their attempt to evaluate the new possibility, some scientists failed to reproduce Arnon's findings altogether;[45] and even those who accepted the data as such (reproduced or not) instead tried to provide alternative explanations for Arnon's results within the framework of the standard hypothesis. One line of criticism was to assume the effect of confounding factors. It was suggested, for example, that Arnon's suspensions might be contaminated with mitochondria (which was, after all, entirely plausible, since it was not easy to get rid of these tiny structures).[46] Alternatively, it was suspected that Arnon's group had formed, as artefacts of their procedure, "a coagulation membrane of some sort which traps [cytoplasmic] enzymes in association with the chloroplast", so that the chloro-

[42]Note how Whatley explained, in retrospect, that, despite all the precautions they took, these "whole" chloroplasts were later found to be leaky, since they lacked the external membrane of intact chloroplasts. This alleged flaw, however, turned out to be fortuitous, as it enabled the substrate of phosphorylation, AMP, to come into contact with the thylakoids, the sites of phosphorylation, which otherwise would have been precluded. See Whatley (1995), p. 18.

[43]Arnon (1954*a*), p. 394.

[44]See also Arnon (1954*b*) in which the findings were reported in more detail. The full evidence for complete photosynthesis in isolated chloroplasts was elaborated in subsequent publications, such as Allen, Arnon, Capindale, Whatley & Durham (1955), Arnon (1955) and Whatley, Allen, Rosenberg, Capindale & Arnon (1956).

[45]See, e.g., Ohmura (1955).

[46]Ochoa put forward this objection against a paper of Arnon's. See the discussion in Arnon (1956) (pp. 307–308).

plasts "may be merely floating around in a cytoplasm which itself contains all the necessary enzymes".[47] In addition to this search for uncontrolled factors in the experimental set-up, some contemporaries were suspicious of the low rates of phosphorylation and carbon dioxide assimilation observed by Arnon et al., which made people doubt the significance of the findings.[48] In later accounts of the discovery, Arnon never failed to mention these critical reactions, not without displaying the satisfaction of having been proven right by history. He also liked to recount how the journal *Chemical and Engineering News* had invited him to give an account of his group's work, "but in the end the editor declined to publish the article because it 'did not pass review by three outstanding authorities in the field".[49] Even the plant physiologist André T. Jagendorf, who would later be one of the first supporters of the photophosphorylation hypothesis, was at first not at all convinced.[50] To some extent, this sceptical attitude was well justified: in view of the experimental difficulties his group experienced, Arnon's proposal, although presented with much fervour and self-confidence, was not the most promising of all the alternative suggestions of the time. Thus, whether *all* photosynthetic processes really did, in fact, take part in the chloroplast remained a controversial issue for some years to come.

However, the fact that there might be a light-induced phosphorylation mechanism was (re-)discovered independently in the same year (1954) by Albert Frenkel. According to his recollections, Frenkel was prompted to study ATP metabolism as a result of working, together with the plant physiologist Allan Brown, on a review of photosynthesis, which he had been invited to write for the journal *Annual Review of Plant Physiology*.[51] While Brown concentrated on those issues related to carbon metabolism, Frenkel covered phosphorus metabolism. He studied intensively, for example, Vishniac & Ochoa (1952*a*) and Vishniac & Ochoa (1952*b*) and decided to spend some time in a research laboratory that focused on related questions. The opportunity to do so arose when Frenkel was awarded sabbatical leave and he arranged to go and work in Fritz Lipmann's laboratory at Harvard Medical School in Boston during the first half of 1954. Lipmann drew Frenkel's attention to the paper by Gest & Kamen (1948), which indicated that there was evidence that ATP was produced in the illuminated cells of *Rhodospirillum rubrum*. The outcome of the research project, which Frenkel initiated thereafter, was reported in a letter to the editor of the *Journal of the American Chemical Society*, the beginning of which reads as follows:

Sir:

[47] See the objection brought forward by Strehler at the 1955 Gatlinburg conference; recorded in Allen, Whatley, Rosenberg, Capindale & Arnon (1957) (discussion of the paper), p. 293.

[48] With hindsight, Arnon believed that this was caused by the fact that the chloroplasts were kept in salt solutions. See Arnon (1987), p. 41.

[49] Arnon (1984), p. 258.

[50] See Jagendorf (1998), p. 219.

[51] See Brown & Frenkel (1953). Frenkel himself has told this story in autobiographical accounts, such as, e.g., Frenkel (1993) and Frenkel (1995). The review was published as Brown & Frenkel (1953).

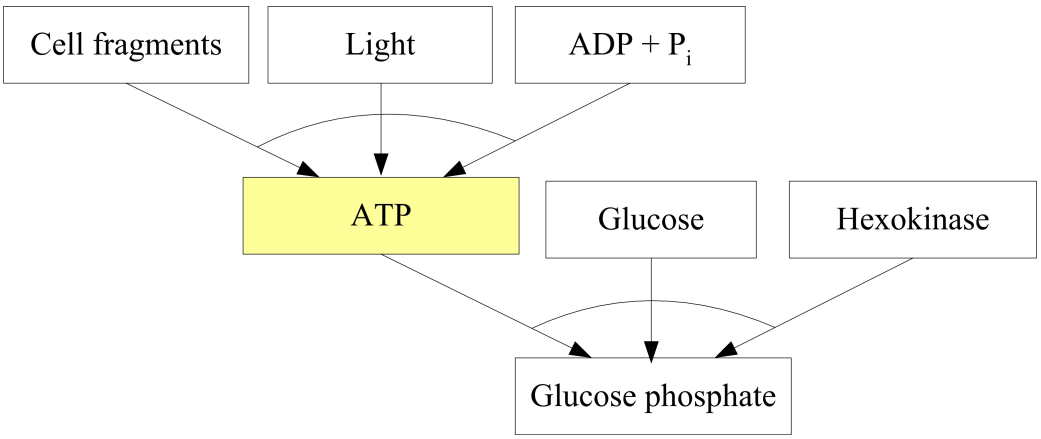

Figure VI.4: small The process of light-induced ATP formation as presented in Frenkel (1954): a suspension of cell fragments, ADP and inorganic phosphate, to which glucose and hexokinase were added, was illuminated. This led to the formation of glucose phosphate (a process which is catalysed by hexokinase and strictly dependent on ATP) and to a decrease in the concentration of inorganic phosphate. From these findings, Frenkel concluded that ATP had been formed.

In the course of a study of phosphorylation with cell-free preparations from *Rhodospirillum rubrum* (strain S-1) it was observed that light induced anaerobically a pronounced disappearance of orthophosphate.[52]

Frenkel was convinced that the phosphate ions disappeared as a direct result of the substantial formation of ATP by bacterial cell fragments, which, he argued, he was able to demonstrate by the fact that he had successfully coupled t his process to the hexokinase-catalysed phosphorylation of glucose. The latter reaction was known to be strictly dependent on the presence of ATP; hence, if it could be shown that this reaction did indeed take place, as Frenkel was able to do, this was proof that the system was able to generate the necessary ATP (see fig. VI.4). When the suspension was centrifuged, all the phosphorylating activity was found in the sediment, which contained the bacteria's chromatophores. Frenkel emphasised that phosphorylation was strictly light-dependent, did not require oxygen and was not inhibited by the typical inhibitors of respiration. Frenkel recalled that, when he submitted this paper, "the editor replied that a paper from Arnon's laboratory had come to his attention which described photosynthetic phosphorylation by isolated chloroplasts". Frenkel cited this paper, which was Arnon (1954*a*), although, according to his own written account, he had not actually seen it. [53] This made many people believe that Frenkel had "confirmed" Arnon's discovery, although his research was done completely independently of the Berkeley laboratory.

It took another three years before any further confirmation of ATP formation in chloroplasts was published – this time by Mordhay Avron and the aforementioned

[52] Frenkel (1954), p. 5568.
[53] Frenkel (1993), p. 110; Frenkel (1995), p. 74.

Jagendorf.[54] However, far from being discouraged by the controversial reception of their results, Arnon's group continued to investigate the capacities of the chloroplast and the interrelation of the different partial processes they had identified: photolysis of water, photosynthetic phosphorylation and carbon dioxide assimilation. These, Arnon and his collaborators argued, were three distinct phases of photosynthesis and each of them was catalysed by a fully independent enzyme system. By 1956, Arnon's group had found striking interdependencies, from which they concluded that the three processes were of increasing complexity:

> Photolysis could be carried out by preparations incapable of photosynthetic phosphorylation and CO_2 fixation. In turn, photosynthetic phosphorylation was found to proceed unimpaired in preparations which could not fix carbon dioxide. CO_2 fixation, however, has been observed only in chloroplast preparations capable of active photolysis and phosphorylation.[55]

These results were supplemented in 1956 by the finding, also made by Arnon's group, that the occurrence of complete photosynthesis outside the cell did not necessarily depend on the structural integrity of chloroplasts.[56] Chloroplast fragments, produced by osmotic shock treatment with water or dilute salt solutions (which prompted the cells to burst), were also able to carry out all three partial processes at surprisingly high rates, as long as the appropriate cofactors were added: "With proper additions, the photosynthetic activity of the reconstituted system, as measured by CO_2 fixation per unit of chlorophyll, was several times greater than that of the intact chloroplasts."[57] Restoring the carbon dioxide fixation required that the membranous fragments were supplied with pyridine nucleotides, ATP and the water-soluble portion of intact chloroplasts. This indicated that the membrane fragments lacked the necessary enzymes for carbon dioxide fixation, which instead seemed to be localised in the stroma fraction of chloroplasts. Photophosphorylation, by contrast, was found to be fully restored in the fragments without the soluble chloroplast fraction, on the mere addition of either magnesium ions (Mg^{2+}), vitamin K, ascorbate or FMN (flavin mononucleotide or riboflavin-5'-phosphate). No oxygen was required for phosphorylation to take place, not even in catalytic amounts. Arnon's group concluded that, since only cofactors had to be added, all the enzymes of photophosphorylation seemed to be contained in the membrane fragments. This was the first of several papers published by Arnon's group that were concerned with the question of the appropriate "cofactors" of photophosphorylation. Not all these papers were consistent with each other, which caused confusion, occasionally consternation and even amusement among Arnon's colleagues. One researcher who was exceedingly sceptical about the importance of specific "cofactors" was Robin Hill, who refused to be impressed by the pertinent work. Hill's long-standing collaborator David Walker vividly remembered the following episode:

> At the time the word from Berkeley was of more and more co-factors. Despite our immense respect for Dan Arnon et alia [sic], we irreverently

[54] See Avron & Jagendorf (1957).
[55] Arnon, Allen & Whatley (1956), p. 458.
[56] See Whatley et al. (1956) and Arnon et al. (1956).
[57] Arnon et al. (1956), p. 462.

> labelled a jar of marmite "Arnon's Reagent" because it worked as well as most compounds that we had tried. In the same spirit Robin suggested "spit, urine and floor sweepings". We shrank from the first two and felt that the third, given Robin's lab, would have been a bit of a forgone conclusion. Even so, there seemed to be a good reason for supposing that almost anything, with an appropriate redox potential, might suffice as a co-factor.[58]

Emerson also had a strong aversion to the way Arnon advertised his findings, notwithstanding their scientific value. On 22 August 1957, Emerson wrote apologetically to his old friend James Bonner: "I'm sorry I'm not getting out to the meetings at Stanford. I would not enjoy Arnon's pompous maunderings about 'photosynthetic' phosphorylation. (As far as I understand what he has done, it is no more than 'photo'phosphorylation.)"[59]

Finally, in 1958, Arnon's group was able to demonstrate that, by making a more considered choice of experimental conditions, such as pH and chloroplast density, the rate of photophosphorylation in isolated chloroplasts, or chloroplast fragments, could be dramatically increased (up to 170 times higher than the rates initially described).[60] The rate of ATP production thus obtained was several times higher than the usual rates of oxidative phosphorylation found in the mitochondrion, and would have compared to forming several high-energy phosphate bonds for each molecule of carbon dioxide that under comparable conditions would have been assimilated. Thus, the phenomenon had definitely reached significant dimensions.

Before I turn to Arnon's finding that there might be different types of ATP formation in the chloroplast, I shall first examine the developments made in a completely different field of research: the work on photosynthetic cytochromes, primarily undertaken by Hill in his laboratory at Cambridge (UK), and the intensive search for a photosynthetic electron transport chain.

3 In Search of a Photosynthetic Electron Transport Chain

3.1 Background

The first thoughts that there might be a stepwise electron transport chain were explored in the study of respiration and related molecules, such as cytochromes, during the 1930s. By 1940, this had led to the idea of a chain of successively oxidised and reduced intermediates. The details of this chain were unknown, although it was strongly suspected that it involved pyridine nucleotides, flavins and cytochromes in the order of their relative oxidation-reduction potentials.[61] The general concept was formulated, for example, by the Harvard-based biochemist Eric G. Ball in 1942 as follows:

> The energy liberated when substrates undergo air oxidation is not liberated in one large burst, as was once thought, but is released in stepwise

[58] Walker (1992), p. 337. For a more detailed (and serious) description of the work going on in Cambridge at the time, see Cambridge University Library, Ms. Add. 9267/ B.275: Programme of Research c. 1959, formulated for the renewal of Fay Bendall's grant (then still referred to as "Miss Myers".

[59] Emerson to Bonner, 22 August 1957, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Bonner, James, University of Illinois Archives.

[60] See Allen, Whatley & Arnon (1958).

[61] On the history of respiration research, see, e.g., Fruton (1972) and Edsall (1974).

> fashion. At least six separate steps appear to be involved. The process is not unlike that of locks in a canal. As each lock is passed in the ascent from a lower to a higher level a certain amount of energy is expended. Similarly, the total energy resulting from the oxidation of foodstuffs is released in small units or parcels, step by step. The amount of free energy released at each step is proportional to the difference in potential of the systems comprising the several steps.[62]

Decisive experimental evidence pointing to the actual sequence of electron carriers in respiration was only provided in the mid-1950s, thanks to the development of rapid and sensitive spectrophotometric techniques introduced by Britton Chance in collaboration with the biochemist G. R. Williams.[63] The underlying notion was that the absorption spectra of biologically relevant molecules, such as cytochromes, flavins and pyridine nucleotides, changed appreciably upon changes in the molecule's oxidation-reduction state. This made the recording of absorption spectra an extremely powerful way of tracing the oxidation and reduction states of certain compounds under certain conditions - for example, when they were supplied with molecular oxygen. It also transpired that these oxidation-reduction processes were somehow linked to the formation of ATP (although the mechanism of this coupling was unknown). Based on their spectroscopic studies, Chance and Williams finally suggested that, in the respiratory chain, electrons were transported in the sequence DPNH, Flavin-H_2, cytochrome *b*, cytochromes *c* and *a*, and, finally, oxygen.[64] Looking back, Chance recalled the frustrating first presentation of their results:

> The responses to the ideas were definitely "not great"; the initial presentation before the American Society of Biological Chemists at San Francisco as a ten-minute presentation was disaster-ridden. The chairman, my good friend A[lbert] Lehninger, apparently misread the clock and told me that I was to sit down after four of the ten minutes allotted for the talk.[65]

However, after the results had been confirmed by several laboratories that had used Chance's dual wavelength spectrophotometer and extended it to the study of living tissue, other scientists started to become interested in the concept. And while Chance and Williams had explicitly refrained from making any comments on photophosphorylation, and its potential coupling to analogous electron transport chains, suggestions construed along these lines soon appeared in print. Even though the principle seemed convincing, and it was generally agreed that there had to be an "uphill flow" from a redox system with a relatively negative potential (O_2/H_2O) to a redox system with a relatively positive potential (CO_2/CH_2O), which was overcome by the absorbance of light energy, this did not yet solve the problem. Rabinowitch pointedly formulated in 1945 (continuing the "lock" metaphor that Ball used in

[62] Quoted in Fruton (1972), p. 387. See Ball (1942) for the original text.

[63] See Chance (2004) for an autobiographical review of the development of the pertinent instruments. Yodh & Tromberg (2000) is a tribute to Chance.

[64] Chance & Williams (1956) provides the celebrated review article of these findings. A retrospective commentary on the latter is given in Chance (1983). See Chance & Williams (1955) and Chance, Williams, Holmes & Higgins (1955) for the earlier papers.

[65] See Chance (1983).

his formulation, and quoted above): "When a canal is built between two bodies of water situated at different levels, the provision of locks cannot be avoided; but whether these locks are constructed at the upper or lower end of the waterway is a purely practical problem."[66] Among the many approaches adopted around 1950 to try and find an appropriate series of electron or hydrogen carriers in photosynthesis was the more detailed study of the occurrence in plants of components that were also involved in the respiratory chain: namely, cytochromes. This was intensively done, for example, in Cambridge (UK).[67]

3.2 Photosynthetic Cytochromes

The 1930s were an enormously decisive period from the viewpoint of understanding cytochromes and their role in metabolism. It was predominantly Cambridge-based biochemist David Keilin who elucidated the action of cytochromes *a*, *b* and *c* as oxidation-reduction catalysts in cellular respiration, since they were reversibly reduced and oxidised by changes in the iron portion of their haeme moieties. Arguably Keilin's most important contribution was to identify the (up to then) mysterious enzyme *cytochrome oxidase* with a specific component of cytochrome a_3, which interacted directly with molecular oxygen. [68] The fact that Hill's work at Cambridge led to cytochrome research being introduced to the field of photosynthesis in the 1940s and 1950s was also hugely influential. Hill's previous studies, which included the far-sighted assumption that photosynthesis might be dependent on oxidation-reduction systems similar to those in respiration, were described earlier in this book, and his close association with Keilin was mentioned as being highly significant in this context (see Chapter III, pp. 150ff.).

It was also stated in Chapter 3 that Hill collaborated with Keilin around 1930 on the isolation of cytochrome *c* from both yeast and muscle tissue, and that even in later years Hill never lost contact with his mentor. The study of the "chloroplast reaction", as Hill insisted on calling the production of oxygen by chloroplast fragments (although everybody else called it the "Hill reaction"), developed at the end of the 1930s into a more in-depth study of the redox capacities of chloroplasts. Hill strongly suspected that cytochromes were involved in these processes as well, and started to explore a little further the presence and function of cytochromes in plants. His first success, which he made with the Indian student Kamala Bhagvat, was to demonstrate the presence and activity of cytochrome oxidase in plant tissues. These same tissues were also shown to contain the well-known cytochromes *a*, *b* and *c*, which were also assumed to be associated with cellular respiration. Interestingly, the cytochrome system was found to be attached to small particles, which could be obtained from a variety of plant tissues – mitochondria, as we know them today.[69] In the course of these studies, Hill realised that leaves and the other green parts of plants had a surprisingly high concentration

[66] Rabinowitch (1945), p. 151.

[67] The first rudimentary ideas on the occurrence of electron transport chains in photosynthesis have been traced back to Katz (1949), Levitt (1953) and Levitt (1954), although none of these were widely received or promoted any further at the time.

[68] For a more detailed account of these discoveries, see, e.g., Mann (1964) and Keilin (1966).

[69] Hill & Bhagvat (1939); see also Bhagvat & Hill (1951) for an elaboration. According to Bendall & Walker (1991), p. 4, this work of Hill's, together with his student (later Professor Kamala Sohonie), "represented the first biochemical study of plant mitochondria".

of haematin compounds (one species of which were cytochromes) – surprisingly high, in view of the relatively low respiratory activity of the tissue. This encouraged Hill, together with Richard Scarisbrick, to embark on a thorough study of the plant's haematin compounds: if respiration only accounted for a minor portion of these compounds, what explained the remainder? This project was started before 1940, but the outbreak of the Second World War forced Hill and Scarisbrick to discontinue their studies, which they were only able to resume in the late 1940s.

The first findings were thus only published in Hill & Scarisbrick (1951), in which the authors reported that three different cytochromes had been obtained in a soluble form from plant tissue. One of these was the well-known cytochrome *c*, while the other two had been hitherto unknown and seemed to be characteristic of plants only. The first, which Hill and Scarisbrick had extracted from the acetone powders of leaves, was tentatively called "cytochrome b_3": found in the green as well as in the colourless parts of plants, it was autoxidisable, did not combine with carbon monoxide and was easily denatured by heat and organic solvents. The second new cytochrome compound, however, was far more exciting: it was only found in the green parts of plants, was not autoxidisable, did not combine with carbon monoxide and was rather stable in the presence of organic solvents (with their denaturing influence). Its absorption properties in intact leaves resembled those of cytochrome *c*, although the bands were much more sharply defined, and there was a characteristic α-band of the reduced component that could be observed at 555 nm. Furthermore, the new cytochrome had definitely more oxidising redox potential than cytochrome *c*. Hill and Scarisbrick called it cytochrome *f*, "(Latin *frons*) on account of the association with the green parts of plants which is indicated by our present observations".[70] In contrast to cytochrome b_3, this compound was found to be rather firmly associated with the chloroplast fraction. It proved, in fact, impossible to extract it in an unmodified form from acetone preparations, although Hill and Scarisbrick found that freshly ground leaves to which ethanol was added (which was a rather drastic treatment!) gave surprisingly high yields. This might be explained, the authors suggested, by assuming that cytochrome *f* was an integrated constituent of the insoluble parts of chloroplasts (which, incidentally, made it very unlikely that it was involved in respiration). Hill and Scarisbrick concluded the paper by stating:

> The occurrence of this specialized cytochrome component strongly indicates the presence of oxido-reduction mechanisms in connexion with chloroplasts which differ both in nature and intensity from those characteristic of the normal respiration of green tissue.[71]

In view of the crude methods available at the time, isolating cytochrome *f*, which, as we know today, is an integrated membrane protein, was a remarkable achievement. In any event, it was the first substantial indication that there were distinct photosynthetic oxidation-reduction processes in which cytochromes played a role. However, many questions were left open, the most pressing one of which

[70] Hill & Scarisbrick (1951), p. 99. See also Bendall (2004) for a review of the history of the discovery and further investigation of cytochrome *f*.

[71] Hill & Scarisbrick (1951), p. 110.

concerned the reality of this proposed cytochrome *f*. After all, it had been isolated only under unphysiological conditions to say the least, so that it was natural to speculate that the whole compound might turn out to be an artefact of preparation that had arisen from the unintentional modification of other haematin compounds in the course of extraction. Therefore, together with Harold Davenport, Hill immediately set out to make an in-depth exploration of the new cytochrome's properties, in particular by comparing it with the better-known cytochrome *c*.[72]

Out of a range of plant species that Davenport and Hill had tested, curled garden parsley in its first year of growth turned out to be the best material source. In the usual down-to-earth type of analysis practised at the time in Hill's laboratory, parsley leaves were puréed in an electric meat grinder, squeezed through a cloth and centrifuged for ten minutes. From the resulting suspension, a satisfactory portion of the compound in question was precipitated. Although the yield was found to depend on several details of the method, the findings were fully affirmative of Hill and Scarisbrick's results. Cytochrome *f* was not an artefact but in all probability a real compound. This was confirmed, only a few months later, by Davenport (1952), in which he reported that the absorption spectrum of cytochrome *f* could be directly observed, without any ethanol treatment, in the intact plastids of etiolated barley leaves (that is, leaves that have green and pale areas).

In addition to its absorption band at 555 nm, the most striking property of cytochrome *f* was its positive redox potential (determined as +365 mV): more positive than the potential of any of the haematin compounds examined up to then. This implied that the reduced state of cytochrome *f* was energetically strongly favoured. In fact, Davenport and Hill were unable to find a system in the leaves capable of oxidising this cytochrome, that is, a system comparable to the cytochrome *c* oxidase in the mitochondrion. In terms of electron transfer, the compound was apparently a dead end; there was, unfortunately, no direct evidence of the metabolic function of cytochrome *f* (which Hill and Davenport might have imagined to be analogous to the central function of cytochrome *c* in respiration). However, Davenport and Hill were still fully convinced that cytochrome *f* did have an important part to play in the photosynthetic electron transport process. Hill and his co-worker Edward F. Hartree stuck to this conviction, which still lacked any evidence, in their comprehensive review of cytochromes in plants in Hill & Hartree (1953). It was only shortly after this review, however, that Hill was able to report the discovery of yet another plant-specific cytochrome:

> The chloroplasts of the etiolated barley leaves were found to contain also a cytochrome *b* component present in amounts definitely larger than that of cytochrome *f*. The *b* component, which here will be designated b_6, was autoxidizable in the chloroplast suspensions and did not combine with carbon monoxide. It was not found possible to remove it from the solid material in an unmodified form. [...] An apparently identical b_6 component was found to be present, together with cytochrome *f*, in *Chlorella*.[73]

In the chloroplast suspensions made from barley leaves, cytochrome *f* was almost immediately and completely reduced on the addition of ferrous iron, while

[72] See Davenport & Hill (1952).
[73] Hill (1954), p. 502.

the newly identified cytochrome b_6 remained in an oxidised state for a surprisingly long period of time; in fact, when the ratio of the ferrous [Fe(II)] to ferric [Fe(III)] iron equalled 1:1, only about half of the cytochrome b_6 had been reduced. The redox potential was thus estimated to be approximately -60mV. "The presence of cytochrome b_6 could account for the experimentally observed reducing properties of chloroplast preparations when illuminated in the presence of certain hydrogen acceptors," Hill suggested.[74] Thus, cytochrome b_6 was considered to be a decisive factor in the Hill reaction. In an illuminated leaf, Hill speculated, cytochrome b_6 might be reduced, while cytochrome f was oxidised: "This represents obviously a definite amount of available chemical energy."[75] By 1954, Hill was clearly hunting for the appropriate components of a potential photosynthetic electron transport chain: the two cytochromes were apparently promising possibilities, although their relationship to each other was far from clear.

Hill had first reported his finding of cytochrome f at the 1952 Gatlinburg conference; and already then had considered it a possibility that this compound might be an intermediate in a photosynthetic electron transport chain. [76] Shortly before, Kamen, by then based at Washington University in St. Louis, Missouri, had also almost unintentionally begun to study the role of cytochromes in photosynthesis. Together with his associate Leo P. Vernon, Kamen observed that bacterial chromatophores of *Rhodospirillum rubrum* extracts showed, upon illumination, a substantial ability to oxidise cytochrome c (which has a more positive redox potential); while in the dark, cytochrome c remained largely reduced (which is its energetically favoured state). The same was found to be true of intact bacterial cells. This was completely unexpected. [77] However, even more astonishing was the finding that bacterial chromatophores also *contained* something that was surprisingly similar to a cytochrome. This compound was present in amounts even higher than the concentration of cytochromes reported to be present in mitochondria; and further investigation demonstrated that the physico-chemical properties of the compound were, as far as Kamen and Vernon could judge, almost identical to those of cytochrome c.

This was a stunning revelation. Nobody had ever suspected that cytochrome c, a key intermediate of respiration, might be present in anaerobe organisms such as *Rhodospirillum rubrum*. It had been considered so unlikely that no one had even bothered to look for it (but even if they had most microbiologists would probably not have had the skills to identify this compound; in 1951 tracing an absorption spectrum was not yet the trivial exercise that it would later become with the advent of fast recording spectrophotometers).[78]

When Kamen spoke to Hill about this discovery at the 1952 Gatlinburg conference, the latter was absolutely thrilled. The presence of cytochrome c and the light-induced cytochrome c oxidising activity in anaerobe bacteria were the first indications that stepwise oxidation processes also occurred in photosynthesis. Hill

[74] Hill (1954), p. 502.
[75] Hill (1954), p. 503.
[76] Cf., e.g., the report of the discussions at Gatlinburg in Hendricks (1953), p. 372.
[77] The findings were published in Vernon (1953) and Vernon & Kamen (1953).
[78] See Kamen (1985), pp. 255–256. In 1952, microbiologists had only just started to use the famous Beckman DU spectrophotometers.

was determined to try out this experiment himself – and *straightaway*; since he had no access to a laboratory in the US, Hill tried to arrange a session at Urbana, Illinois, where he, together with Kamen and Emerson, could repeat the oxidation of cytochrome *c* using illuminated *Rhodospirillum rubrum* cells. Hill raised the issue in a letter to Emerson on 1 November 1952, emphasising the importance he attached to the experiment: "We are nearly at the point of finding whether it is one big step or four little ones in the reducing mechanism"; to which Hill added, with regret: "They [Kamen and Vernon] can't do it yet with *Chlorella* but this is not serious at present." [79] This letter to Emerson was closely followed by a second, more definitive one, written the following day:

> I have much on my conscience to expose. [...] I suggested to Kamen that we might all meet in your laboratory and really see the oxidation of cytochrome *c* when I come back from Stanford. He is almost persuaded to publish this result and if we could all do the experiment together, then perhaps it might happen. I think it is a landmark and if it is delayed until further complications ensue, they will drag it out until the wonderful inspiration it might give will be blurred. I will try to get a sample of [cytochrome] *c* from our lab because it might be different from the commercial [cytochrome] *c* grown here in some way. One of the people in [Ernest F.] Gale's lab in Cambridge is working on *R. rubrum*, but I do not think this work directly affects what he is doing. This would mean that you need not worry about getting bugs grown – though of course it would be good to have an experiment with independent materials – for then they could be free of doubts about publishing. [...] (If we could look for cytochrome *f* in *Chlorella* and in *Rhodospirillum* at the same time, it would be fine.)[80]

However, Emerson was clearly not as well informed as Hill believed. As one can take from Emerson's answer, he had difficulty in immediately grasping the full implication of Kamen's findings and, thus, the reason for Hill's excitement. Eventually, Emerson came up with the following hypothesis, which he cautiously asked Hill to confirm:

> Tom Punnett, [Stanley] Holt, Rabinowitch, and I have discussed your suggestions about cytochrome *c*, *f*, *Rhodosp. rubrum*, and *Chlorella*. It took us some time to reach agreement on the interpretation of your letters. Doubtless you will think us very stupid, but please let me ask if we understand you. Is it like this: You're thinking that if cytoch. *c* is oxidized under *anaerobic conditions* by *Rhod. rub.* juice, probably the (electron or H atom) is transferred toward PGA or DPN or TPN rather than toward O_2. You would like to regard this as evidence for a *stepwise* increase in energy from water toward PGA, maybe by these steps: H_2O – cytochr. *f* – cytochr. *c* – cytochr. *a* – ... – PGA or DPN or TPN.
>
> If this is also the pathway (or something like it) in *Chlorella*, then it should be possible to find the cytochr. *c* oxidation in *Chlorella* juice. Since Kamen and Vernon have failed, so far, to find it, maybe they are not sure that in the

[79] Hill to Emerson, 1 Nov. 1952, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Hill, Robin, University of Illinois Archives.

[80] Hill to Emerson, 2 Nov. 1952, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Hill, Robin, University of Illinois Archives. Ernest F. Gale, a British microbiologist, was a friend and colleague of Hill's at Cambridge; see for more information, e.g., Reynolds (2007).

> case of *Rhodo rub.* the oxidation really goes toward reduction of PGA rather than the other direction, toward reduction of oxygen. Is absence of oxygen in their experiment their only evidence that the hydrogen (or electron) moves toward PGA? In any case, I hope Davenport can send some [cytochrome] *c* in time, and we will be prepared with *Rhod. rubr.* cells. A letter came today from Martin Kamen, saying they have found oxygen is necessary. This should not demolish our hopes, it may still prove to be possible in the absence of O_2, but it shows (if I have interpreted your letters correctly) that exclusion of oxygen is absolutely essential, if oxidation of cytochr. *c* is to have any significance.
>
> I will write Kamen, and see if he and Vernon would like to come up here during your next stop with us. I'll offer them both travel expenses. How much *Rhodosp. rub.* do you think you want? We shall have plenty of *Chlorella*, if you think it worth trying.[81]

The experimental session eventually took place in December 1952. Kamen described the session as follows:

> While there [at Urbana], we showed him [Hill] our data on photo-oxidation of mammalian and bacterial cytochrome *c* and with his collaboration performed some experiments on the quantitative relation between amounts of cytochrome *c* oxidized and amounts of oxygen used. We published our findings shortly thereafter, including a speculation that bacterial photosynthesis could proceed through reactions in which compounds other than water donate hydrogen and were required for growth might be photo-oxidized directly rather than via photosynthetic fission of water.[82]

This finding by Kamen and Vernon met with considerable disbelief; however, time was on their side, and when it transpired that the compound was very similar, but not identical, to the usual cytochrome *c*, Kamen and Vernon felt entitled to call the compound (at Keilin and Hill's suggestion) cytochrome c_2. This was only the first of a whole series of additional cytochrome *c* variants that would be detected in a range of other organisms. Kamen and Vernon themselves were able to find a *c*-type cytochrome in the strictly anaerobic bacterium *Chlorobium limicola*: this was highly significant, since there was no alternative aerobic mechanism, as in *R. rubrum*, that might have accounted for the presence of cytochrome *c*.[83]

[81] Emerson to Hill c/o Stacy French, Stanford, on 10. Nov. 1952, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Hill, Robin, University of Illinois Archives. It was in the same letter that Emerson added, with some exasperation: "I must try and write to C.P.W. [Charles P. Whittingham] about the Gatlinburg meeting. I agree with you that the pace was exhausting, and the Sheffield meetings were much more profitable. How I wish I could have made some more constructive comments about the quantum yield controversy!"

[82] Kamen (1985), p. 256.

[83] In a letter of 1954, Kamen reported to Hill the results of an extended research stay he had enjoyed at van Niel's laboratory at the Hopkins Marine Station in Pacific Grove : "To summarize: All the photosynthetic bacteria, regardless of their aerobic or anaerobic character contain a cytochrome *c* component. [...] All the bacteria have a photo-oxidase, a thermal oxidase and a DPN-linked reductase for their cytochrome *c*." Cambridge University Library, Ms. Add. 9267/J.75. Kamen to Hill on 25 September, 1954. It was in the same letter that Kamen wrote: "There is ample evidence from the bacteria that the hematin compounds are involved directly in light metabolism. It appears to me that it is time to explore, at the speculative level, schemes for coupling the cyto components, in a modified H-transfer system to the light absorption." Kamen

Shortly thereafter, laboratories in England and Japan also reported the presence of cytochrome *c* in anaerobic bacteria. This was much to the satisfaction of Kamen, and also of Hill:

> Thus, in the short span of a year, the dogma that cytochrome *c* was uniquely involved in the reduction of oxygen by mitochondria, and not in metabolism in the absence of oxygen, was decisively disproved. The particular cytochrome *c* that occurred in air-breathing organisms and was localized in their energy-transducing organelles, the mitochondria, was a critically important catalyst for biological oxidation. It could now be seen, however, that it was only one of a large family of similar heme proteins with the same iron porphyrin as part of the active catalyst, reactive generally in redox metabolism not only in aerobes but also in anaerobes.[84]

Hill also continued to pursue the phenomenon, trying to make sense of the different cytochromes and their functions, specifically in photosynthesis, which explains why he was so interested that Kamen and Vernon's phenomenon could also be found in *Chlorella*. On 10 June 1953, Hill informed Emerson about some successful experimental work of his new assistant, Donald H. Northcote, who had been able to identify the spectral "fingerprint" of cytochrome *f* in *Chlorella* cells – which Hill and Davenport had failed to see – as well as find evidence for the reduction of photochemical dyes in *Chlorella*. Hill was delighted: "The activity was not very great: ∼1/4 average of the leaf preps we use. Next I must see if it will produce O_2. Still it looks hopeful and don't you think we should publish this 1st 'activity' in smashed *Chlorella* soon?" Hill then added:

> I have just seen Vernon's paper in the Archives on [cytochrome] *c* in *Rhodospirillum*. I was a little disappointed that they only give this violent method of isolation when we found together that it came out with buffer from the cold acetone preps. But I still owe Leo a letter and will ask him about it. A wonderful week it was. I still think about it and how much you did to make it all possible. [85]

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then described in detail how cytochromes might be involved, which he hypothesised from his knowledge of respiratory processes. Looking back, Kamen himself deeply regretted that, despite having mulled over photosynthetic electron transport chains so intensely, he had not succeeded in discovering bacterial photophosphorylation: "The notion that it would be possible to make ATP from ADP and phosphate simply by irradiating chromatophores with ADP and phosphates struck me as simplistic." Therefore, he set out, together with Vernon, on the search for complicated systems enzymatically coupled to the Hill reaction. "Meanwhile, Albert Frenkel [...] tried the simple experiment and found it to work!" Kamen (1989), p. 142; for the successful discovery, see the aforementioned Frenkel (1954).

[84] Kamen (1985), p. 260.

[85] Hill to Emerson and Tom Punnett, 10 June 1953, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Hill, Robin, University of Illinois Archives. Note that Emerson was rather indignant that anybody would wish to have their discovery published at such an early stage. He wrote to Hill on 4 February 1953: "Rabinowitch showed me a manuscript from Kamen and Vernon, submitted to him by the editors of *Archives of Biochem.* for editorial review. It contained a description of our manometric cytochrome experiment, and acknowledgment of the work done in Urbana. I must admit I was surprised that Martin should feel so confident in those results! I suppose biochemical experiments are more conclusive than whole-cell experiments. My own results always seem to me equivocal, and open to several different interpretations. I always want to do them over lots of times in lots of different ways, before I can possibly make up my mind

Two weeks later, Hill was even more delighted:

> Yesterday I had some luck, and found that there was lots of cytochrome *f* in a sample of broken *Chlorella pyrenoidosa* cells. [...] This is very *preliminary*. I hope to get more accurate data for this now. I wish you could have seen it, but hope that you may have already convinced yourself that the cells *do* contain [cytochrome] *f*! It *would* have been odd if they didn't – there's lots of other haematin as well as far as we saw.[86]

3.3 Ferredoxin

In parallel to the investigation of cytochromes, another line of research that Hill and Davenport pushed ahead was the search for the natural hydrogen acceptor in chloroplasts.[87] Artificial acceptors that were added to chloroplast suspensions somehow seemed to act as the substitutes for a distinctive part of the chloroplasts' oxidation-reduction system, which was lost or, at least, inactivated when the chloroplasts were isolated . These lost or inactivated elements had to be found if one wanted to learn more about the redox system in the intact chloroplast. A first step in this direction had already been made in 1949, in studies undertaken by Davenport. He had demonstrated that, although suspensions of washed chloroplasts were unable to produce any molecular oxygen without the additional supply of hydrogen acceptors, the same chloroplasts happily released substantial amounts of oxygen, measured in terms of methaemoglobin reduction, when they were suspended in an aqueous extract made from acetone-treated leaves.[88] However, at the time Davenport was unable to identify any specific compound in the extract that could induce this reduction in capacity.

As can be taken from Hill's correspondence, the question was of considerable concern to him and his collaborators in the following years, and it still took them a couple of years after Davenport's first attempt before they were able to obtain any substantial results. Although Hill himself was fully convinced that, in the end, photosynthesis would turn out to function in a stepwise process similar to respiration, for a long time there was no compelling evidence to support this assumption. In October 1951, Hill wrote to French:

> We are still continuing the work on the methaemoglobin factor; it is not very stable so that our progress is bound to be slow. As you know one of our main concerns is how the energy is to be applied to the biochemical systems and now there does seem to be a matter of two alternatives: one big hitch or

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to write anything up for publication. Seeing Martin go ahead with such self assurance makes me feel very incompetent, especially because my own experimental work seems to progress at such a snail's pace, always against the resistance of stubborn difficulties which are only to be removed by brute labor, mostly re-construction of such things as light sources." Cambridge University Library, Ms. Add. 9267/J.54. Emerson to Hill on 4. Feb., 1953.

[86] Hill to Emerson and Tom Punnett, 23 June 1953, Robert Emerson Papers, 1923-61, Record Series 15/4/28, Box 1, Folder: Hill, Robin, University of Illinois Archives. Hill added, alluding to Calvin's thioctic acid theory, which was still being hotly debated at the time: "Is there any more news about Calvin splitting the S-S of Lipoid? – that is, in the cell or biochemically. It really was an amusing idea & hope it's not really too good to be true."

[87] For accounts of the discovery of what eventually became known as "ferredoxin", see, e.g. Walker (2002), Besse & Buchanan (1998), Bendall (1994) and Arnon (1988).

[88] Davenport, H. E. 1949; Proc. Roy. Soc. B, 136, 281.

> a lot of little ones. Franck has always seemed to favour the one big change so I sent him our papers in the hope of finding out what he would think of this other point of view. The *total* amount of experimental evidence seems to leave the question of the two alternatives quite neutral as far as I can see.[89]

The "methaemoglobin factor" that Hill mentioned in the letter was the focus of a paper that Davenport, Hill and Whatley published in the *Proceedings of the Royal Society* in 1952 (that is, before Whatley went to work with Arnon at Berkeley). However obscure the general path of photosynthesis still was, the authors of this paper were convinced that they had identified at least one of the natural hydrogen acceptors in the chloroplasts, which they preliminarily called "the methaemoglobin reducing factor" (MRF), after its capacity to reduce methaemoglobin (obtained from whale muscle, as can be taken from the acknowledgement) to its oxidised form.[90] According to the authors' observations, washed chloroplasts were able to reduce methaemoglobin in the light, when the soluble fraction of the chloroplast suspension was added; in the dark, by contrast, no reduction occurred. The rates observed in the light were the same as had been found earlier with acetone extracts of leaves and comparable to the usual rate of the Hill reaction with artificial hydrogen acceptors. This made it highly probable that the same reaction was being observed. The factor in question seemed not to be present in the non-green parts of the plants. Davenport, Hill and Whatley concluded from these findings "that the factor is reduced directly by the illuminated chloroplasts, that oxygen is produced in the process, and that the factor acts in the sense of a catalyst for the reduction of methaemoglobin in the illuminated system".[91]

The compound's behaviour displayed some similarities to the hydrogen donor of the cytochrome oxidase system in respiration; in particular the rapidity of the factor's reaction with illuminated chloroplasts suggested, the authors thought, that the factor was immediately concerned with the main function of the chloroplasts, even though they had no direct evidence to support this assumption.[92] However, Davenport et al. did not hesitate to underline the general coherence of this observation with the finding of cytochrome *f*, which was reported in the same issue of the *Proceedings*:

> The object of this and the preceding investigation [Davenport & Hill (1952)] has been an attempt to characterise systems concerned with hydrogen transport which may be found in the green leaf as distinct from the other parts of the plant. The presence of a soluble protein agent transferring hydrogen from the illuminated chloroplast to an added reagent, methaemoglobin, again indicates the partial analogy, previously referred to in connexion with cytochrome *f*, between the chloroplast and the respiratory system of catalysts

[89] Cambridge University Library, Ms. Add. 9267/J.58. Hill to French on 6 October, 1951. The same concern was expressed in a letter from Hill to Whatley, on 7 October 1951: "As you may gather my concern now is to find out whether the light causes one big chemical reaction or lots of little ones. During the summer the latter seemed the most satisfying but as you can imagine when one comes to the minute physico-chemical details one can get in a tangle." Cambridge University Library, Ms. Add. 9267/J. 116.

[90] See Davenport, Hill & Whatley (1952). The acknowledgement reads: "We are very grateful to Dr J. G. Sharpe for his help in supplying us with whale muscle." (p. 358)

[91] Davenport et al. (1952), p. 346.

[92] Davenport et al. (1952), p. 357.

> that seem generally to be associated with mitochondria in aerobic cells. It is in the direction of electron transfer with reference to molecular oxygen that the two different systems are seen to be in greatest contrast. The methaemoglobin reducing factor may be regarded as reacting with the illuminated chloroplast in a sense opposite to a cytochrome reductase reacting with the insoluble respiratory cytochrome oxidase system.[93]

More precise information could not be given at the time, neither on the physiological function of the factor nor on its material identity. It was only years later, in 1960, that Davenport and Hill were able to isolate the actual compound, from leaves as well as from *Chlorella* cells, and characterise the MRF as a non-haeme protein of rather low molecular weight and of deep reddish brown colour. By then, it had been established that the MRF could also catalyse the reduction of a range of other haeme proteins, including cytochromes c and b_3; strangely enough, the factor seemed not to catalyse the reduction of any artificial electron acceptor such as ferricyanide.[94] The redox potential of methaemoglobin was around +100mV, so that the factor's position in a potential electron transport chain was suspected somewhere "below" cytochrome f (that is, more to the negative), while it was unclear how it was related to the redox potential of oxygen. At the time, there was no reason to assume that the MRF would be the agent of the light-induced reduction of TPN (which was later found to be its actual function). As Hill's biographer Derek Bendall pointed out: "The crucial experiment which would have shown that the 'met factor' (ferredoxin as we now know it) was, what he [Hill] was looking for, was never carried out" – although Hill would have had the chance to do so: Almost certainly he possessed a sample of NADP [i.e. TPN] but there is a suspicion that he regarded it as too precious actually to use."[95]

While the MRF was being investigated at Cambridge, a few years later, in 1956, the biochemist Anthony San Pietro and his associate Helga M. Lang, at the Johns Hopkins University in Baltimore, Maryland (US), observed that in concentrated chloroplast suspensions (as well as in diluted suspensions when supplemented with chloroplast extract) reduced pyridine nucleotides (DPNH and TPNH) accumulated, without any "trapping" enzyme system having been added.[96] San Pietro and Lang suggested that "the reduction is an enzyme-catalyzed reaction", and that "the enzyme is highly specific for the intact dinucleotide structure".[97] Later in 1958, San Pietro and Lang were able to isolate a soluble protein from spinach leaves that seemed to be the agent of these reductions; they consequently named it after this capacity: "**p**hotosynthetic **p**yridine **n**ucleotide **r**eductase", or PPNR for short. This, San Pietro and Lang believed, was the decisive enzyme that catalysed the light-induced reduction of pyridine nucleotides (it was later found to act specifically on

[93] Davenport et al. (1952), pp. 357–358.

[94] See Davenport & Hill (1960).

[95] Bendall (1994), p. 159.

[96] Note that, when it was discovered, in 1951, that DPN and TPN were reduced by chloroplasts, the reduced compounds had only been demonstrated indirectly, by means of coupling the process to the enzymatic production of malate. This was found by San Pietro and Lang to be unnecessary, provided that the conditions were appropriately chosen.

[97] San Pietro & Lang (1956). See also Kresge, Simoni & Hill (2005) on the work done by San Pietro and his group, and Pietro (2008) for an autobiographical account.

TPN).[98] Still, in 1958, San Pietro and Lang were unaware of the earlier findings by Davenport et al. (or considered them as completely irrelevant to their work), so that they did not even discuss the possible relationship between PPNR and the MRF.

A third soluble factor identified in aqueous extracts of spinach leaves, which also catalysed the reduction of TPN by isolated chloroplasts, was reported in 1957 by Arnon's group at Berkeley, and named, according to its displayed capacity, the "TPN-reducing factor". It was noted that this factor was not required for the production of oxygen in those reactions in which TPN was not involved, that is, for example, when ferricyanide or other Hill reagents were added.[99] This latter feature very much resembled the behaviour of the MRF. Yet, while Hill and Davenport, based on their experiments, had no reason to suspect that the MRF had anything to do with the reduction of pyridine nucleotides, there was likewise no reason for Arnon and his colleagues to suspect that there was any connection between their newly found protein and the factor described at Cambridge.

Thus, by 1960, three different electron acceptors of a protein nature had been independently described, and they all seemed to be present in the soluble fraction of chloroplasts: the MRF, PPNR and the TPN (NADP)-reducing factor. However, slowly evidence began to accumulate that these factors had striking resemblances and, thus, might be closely related to each other: all of them contained non-haeme iron; they had similar (but not identical) absorption spectra in the visible region; they had comparable redox potentials and they were devoid of flavin.[100] By 1959, Davenport had found that the MRF and PPNR were interchangeable in their effects, that is, they both catalysed the photoreduction of either methaemoglobin or TPN in the chloroplast – and suspected that the two compounds might eventually turn out to be one and the same. Furthermore, Davenport reported that more reduction took place when ADP, inorganic phosphate and magnesium ions were added to the reaction mixture.[101] Hill was exceedingly pleased with this discovery, as can be taken from his annual departmental report for the academic year 1958/59:

> This result [obtained by Davenport, that the MRF was identical to PPNR] would dispose of a whole range of problems. The presence in the plant of a very active H-acceptor for the chloroplast we have known for many years, but until now it has not been possible to fit it in with generally accepted present views on the mechanism of photosynthesis.[...] [T]he original isolation of the active 'methaemoglobin reducing factor' now finally supplies an efficient link between the light driven reactions and the path of carbon in photosynthesis as elucidated by Calvin. I had for some time suspected a relation between the San Pietro and Lang factor and the methaemoglobin factor; we had indeed planned experiments for this summer in this connection. But I had not been so optimistic as to imagine that the two factors would have turned out to be one and the same substance.[102]

[98] San Pietro & Lang (1958).
[99] Arnon, Whatley & Allen (1957).
[100] See Pietro (2008), p. 191.
[101] Davenport (1959) and Davenport (1960).
[102] Cambridge University Library, Ms. Add. 9267/D. 10. In this report Hill also mentioned that he had been working with Fay Bendall on "the action of oxidation reduction reagents required to establish the hydrogen transport necessary for photophosphorylation".

It was in Tagawa & Arnon (1962) that the reducing factors were finally recognised as belonging to the same protein family: not only did the three factors resemble one another; they were also similar to an iron-sulphur protein that had recently been isolated from the prokaryote organism *Clostridium pasteurianum*.[103] This latter protein, named "ferredoxin", was found to mediate the transfer of hydrogen to hydrogenases. Tagawa and Arnon demonstrated that the effects of the MRF, PPNR and the TPN-reducing factor were fully comparable to the effects of ferredoxin, although the compounds differed slightly in their activities, colours and absorption spectra. In view of these findings, Tagawa and Arnon suggested that the term "ferredoxin" be extended to a whole family of compounds that also encompassed the proteins previously found to be involved in the photoreduction of TPN in chloroplasts. This was generally accepted, and ferredoxin is listed to this day as an essential element in the photosynthetic electron transport chain.

3.4 Model Suggestions

Arnon's First Model

At the same time, scientists continued their attempts to model a photosynthetic electron transport chain. Few people challenged the principal idea or the notion that, most probably, upon illumination chlorophyll became the donor of high-energy electrons, which were then transferred to acceptor molecules and utilised to form reducing equivalents or ATP. The exact identity of these acceptor molecules was still unclear, however.

One suggestion came from Arnon's laboratory at Berkeley, when, in 1956, the group tried to explain the phosphorylation phenomena that had been observed in chloroplasts with an appropriate oxidation-reduction scheme of potential intermediates. [104] The original scheme is reproduced in figure VI.5; a reconstruction in the notation of causal graphs is given in figure VI.6. In this model, the photolysis of water was identified as the reaction in which light energy was first converted into chemically usable energy. This explained why photolysis was a prerequisite for both photophosphorylation and carbon dioxide assimilation. The latter two processes were thought to be in competition with each other: the photolysis of water was either linked with the formation of ATP (whereupon the water molecule was immediately restored) or with carbon dioxide reduction, upon which the oxygen of water was released as a gas, while the hydrogen was used in the reduction process. (This was in accordance with the suggestion made fifteen years earlier by van Niel that the primary photochemical process, namely, the splitting of water into [H] and [OH], was the same in all types of photosynthesis, oxygenic and anoxygenic, although in the latter case, the water molecule was immediately restituted. [105]) Each of the two processes thus required a separate expenditure of light energy. The authors then formulated the following hypothesis on the details of the phosphorylation process and the underlying electron transport chain:

> [I]t is envisaged that in photosynthetic phosphorylation the recombination of the products of photolysis of water proceeds in several successive steps,

[103] See Mortenson, Valentine & Carnahan (1962).
[104] See Arnon et al. (1956).
[105] See, for this suggestion, e.g., van Niel (1941).

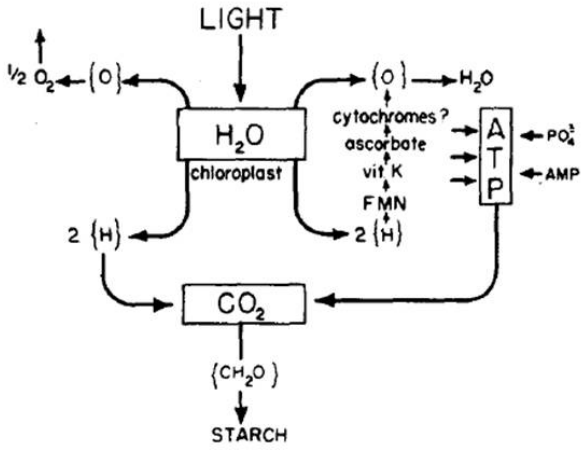

Fig. 8. Scheme for photosynthesis by isolated chloroplasts. Photolysis of water (center) leading either to ATP synthesis and the reconstitution of water (right) or to CO_2 reduction (below) linked with oxygen evolution (upper left).

Figure VI.5: The photosynthesis model published in Arnon, Allen & Whatley (1956), p. 458.

> which together constitute an "electron ladder" analogous to that discussed for respiration [...]. Of the catalysts of photosynthetic phosphorylation Mg^{++} probably has a function in the transfer of phosphate, whereas FMN, vitamin K, and ascorbate could serve as electron carriers in the "electron ladder" shown in Fig. 8 [...]. The identity of the electron carriers above ascorbate is unknown, but they may very likely prove to be components of a cytochrome system.[106]

Although the individual steps differed from those envisaged for respiration, the principle was the same. Light energy was used to effect a "splitting" of a water molecule into [H] and [OH]; the "re-unification" of these molecules was an exergonic process, the free energy release of which was used to build up ATP molecules. Possible steps on the "electron ladder" included the cofactors that Arnon's group had found to be necessary for photosynthetic phosphorylation to occur: FMN, vitamin K and ascorbate; while they additionally (and hypothetically) included cytochromes to cover the final step of the transport chain.

Arnon's Second Model

This scheme was not to last very long, though. In 1957, Arnon's group made several confusing observations that could not be reconciled with their hitherto

[106] Arnon et al. (1956), p. 259.

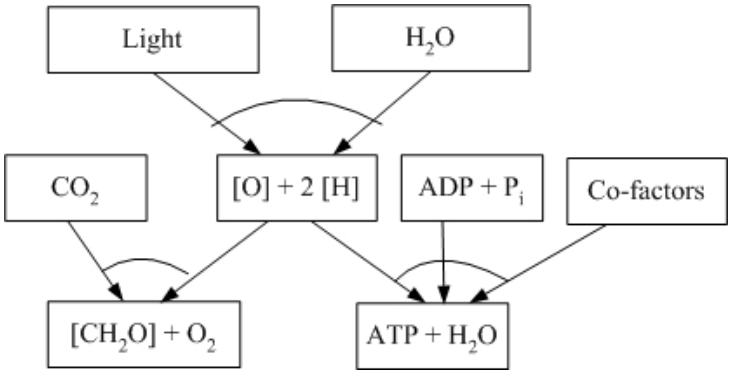

Figure VI.6: Reconstruction of the first photosynthesis model, published in Arnon, Allen & Whatley (1956), p. 458.

concept of photophosphorylation.[107] The first surprising result was that, under certain conditions (an alkaline pH value, a rather low chlorophyll concentration level and ADP instead of AMP as substrate), TPN acted as a significant catalyst of photosynthetic phosphorylation: when TPN was added to isolated chloroplasts, it was immediately reduced, while at the same time substantial amounts of oxygen developed, in accordance with the following reaction:

$$2\mathrm{TPN} + 4\mathrm{H_2O} \xrightarrow[\text{light, chloroplast}]{} 2\mathrm{TPNH_2} + \mathrm{O_2} + 2\mathrm{H_2O} \tag{VI.1}$$

Although this reaction had been known for several years already, it turned out that, in the presence of ADP and P_i, this reaction was found to be coupled to the formation of 2 moles of ATP. Arnon, Whatley and Allen were particularly struck by the stoichiometry of the participating components, which they found to be highly significant; namely, that "the synthesis of 2 moles of ATP accompanies the generation of four hydrogen equivalents, which are required for the reduction of 1 mole of CO_2 to the level of carbohydrate".[108] Formulated as an equation, the overall process was the following:

$$2\mathrm{ADP} + 2\mathrm{P}_i + 2\mathrm{TPN} + 4\mathrm{H_2O} \xrightarrow[\text{light, chloroplast}]{} 2\mathrm{ATP} + 2\mathrm{TPNH_2} + \mathrm{O_2} + 2\mathrm{H_2O} \tag{VI.2}$$

Thus, the light quanta absorbed seemed to be used simultaneously to produce reducing equivalents *and* to form ATP, although in the model described above it had been assumed that these processes were competing with each other for the light quanta. Arnon's group concluded that they had hit upon a different type of photophosphorylation that had so far not been accounted for:

[107] The first published account was Arnon et al. (1957), while Arnon, Whatley & Allen (1958) provided a more comprehensive overview.
[108] Arnon et al. (1958), p. 1029.

> Carbon dioxide fixation remains, as before, at the apex of this hierarchy [of the three partial processes: photolysis of water, photosynthetic phosphorylation and carbon dioxide fixation] and requires the participation of all three groups of enzymes. But photolysis of water is now no longer regarded as resulting either in the synthesis of ATP or in the reduction of CO_2. Adenosine triphosphate [ATP] synthesis is coupled with the formation of the reductant ($TPNH_2$) required for CO_2 fixation. Thus, the same light quanta which accomplish the reduction of TPN also bring about the synthesis of ATP and generate the assimilatory power needed for the conversion of CO_2 into carbohydrates or analogous end-products of photosynthesis.[109]

It was found that TPN was much more favoured than DPN; and the most interesting fact was that this kind of phosphorylation occurred without the presence of any additional cofactors, such as vitamin K or flavin mononucleotide FMN (since it had been demonstrated, in the meantime, that only one of them had to be added, and not both, as Arnon's group had previously believed.[110]) However, when either of these two cofactors *was* added, the resulting picture changed dramatically: "Phosphorylation was sharply increased, whereas oxygen evolution and the accumulation of reduced TPN were abolished."[111] Thus, all the energy was again channelled into ATP synthesis, just as it had been found earlier. The most direct explanation of these results was, Arnon and his collaborators surmised, that the addition of FMN or vitamin K to the reaction mixture initialised again the already well-known path of ATP formation reported earlier, which precluded the reduction of TPN. In order to differentiate between the different types of phosphorylation, Arnon, Whatley and Allen decided to call the first type "cyclic" photophosphorylation, since all the electrons were kept within the system, while the alternative process, in which the reduction of TPN was coupled to ATP formation, was named "non-cyclic" photophosphorylation.

This differentiation was taken up and elaborated in Arnon (1959), a comprehensive summary of the research done by the group during the 1950s: cyclic photophosphorylation was described as the production of ATP only, without any reducing power being accumulated, while non-cyclic photophosphorylation yielded the actual "first" products of photosynthesis: reduced TPN and ATP, both of which were required to accomplish the reduction of carbon dioxide to sugar phosphates. It was believed that the purpose of cyclic photophosphorylation was to provide additional ATP: based on recent research work carried out by his group, Arnon (1959) thought that the ATP produced in the non-cyclic version was insufficient for the carbon reduction process.[112]

Arnon believed that, in cyclic photophosphorylation, a continuous energy input by incident light was necessary to raise the electrons to a higher energy level, while the "downhill" transport could proceed via two alternative enzymatic pathways, one of which involved FMN as an intermediate carrier and the other required vitamin

[109] Arnon et al. (1958), pp. 1029–1030.
[110] See Whatley, Allen & Arnon (1957).
[111] Arnon et al. (1958), p. 1030.
[112] Details of the need for the sufficient and well-balanced excitation of both cyclic and non-cyclic photophosphorylation were published in Trebst, Manuel & Arnon (1959) as well as in Losada, Trebst & Arnon (1959); both were confirmed in Trebst, Losada & Arnon (1960).

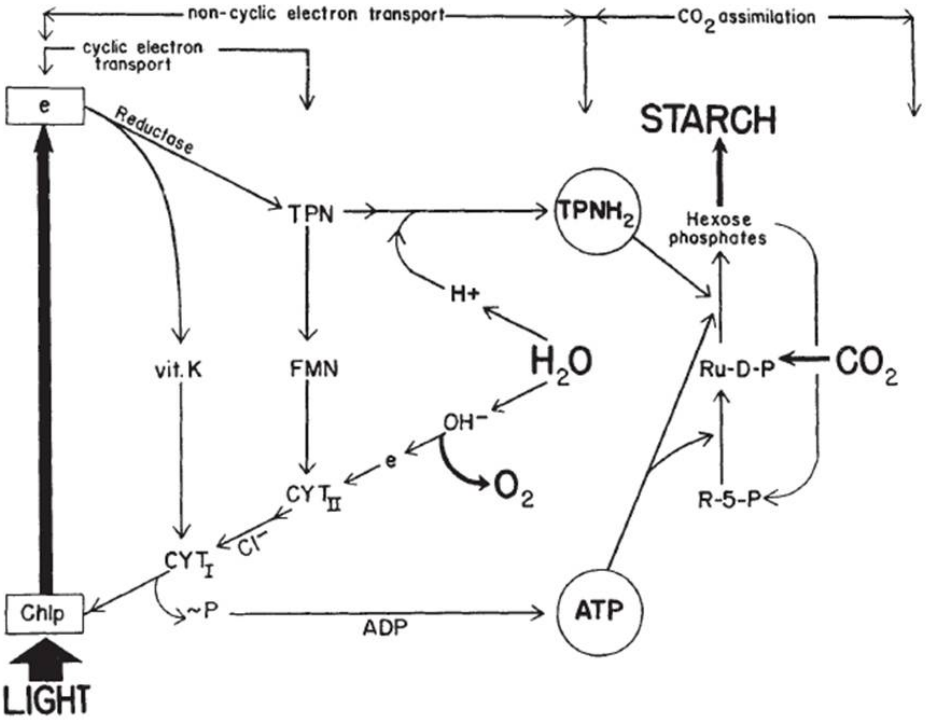

Figure VI.7: The photosynthesis model published in Arnon (1959), p. 14.

K. It was assumed that, along the vitamin K pathway, two electrons expelled from chlorophyll were transferred to vitamin K, thereby reducing the latter; the reduced vitamin K was, in turn, re-oxidised by a cytochrome component, which then donated electrons back to the chlorophyll. Phosphorylation was thought most probably to be coupled to the oxidation of the terminal cytochrome component, and a second phosphorylation event, coupled to the re-oxidation of vitamin K, was not precluded either. The pathway via FMN was conceptualised as a modification of the vitamin K pathway, and in this case TPN and two cytochrome components were assumed to be involved (see fig. VI.7; the process of cyclic photophosphorylation is marked at the top as involving only the processes on the far left of the figure).

Non-cyclic photophosphorylation was thought to involve the same primary photochemical reaction and the same phosphorylating site, namely the oxidation of the terminal cytochrome. In figure VI.7 this is depicted on the left-hand side, where of the two arrows issuing from Cyt_I one leads to the chlorophyll (Chlp) and the other to "$\sim$P". This was an entirely plausible assumption – it appeared highly unlikely that the two mechanisms should travel along completely different paths. The main difference concerned the fate of the electrons: in non-cyclic photophosphorylation the electrons did not return to the chlorophyll, but were removed from the system by the reduction of TPN. The chlorophyll, in turn, was replenished again "by an interaction between hydroxyl ions (or water) and a cytochrome component peculiar

to the photosynthetic apparatus of green plants but absent in photosynthetic bacteria". [113]

However, when explaining non-cyclic photophosphorylation in this vein, one had to deal with the fact that the direction of the general process was the exact reverse of that in oxidative phosphorylation: while in the latter process oxygen was consumed, in non-cyclic photophosphorylation, oxygen was produced. In oxidative phosphorylation, pyridine nucleotides (DPNH) were oxidised, while in non-cyclic photophosphorylation the analogous pyridine nucleotides (TPN) were reduced. This caused considerable consternation and worry among photosynthesis researchers. David A. Walker, then a postdoctoral student of Hill's at Cambridge, remembered how in the late 1950s he had battled to understand fully the curious phenomena of photosynthetic phosphorylation that were coming out of Arnon's laboratory. He found the results confusing to say the least:

> Cyclic [photophosphorylation] was exciting enough but non-cyclic was positively mind boggling. Like others of my generation, I had become accustomed to the idea that the oxidation of reduced "DPN" was accompanied by the esterification of ADP to yield ATP. Conversely, ATP formation accompanying "TPN" reduction [...] seemed remarkably like getting water to run up hill.[114]

The mechanism of photophosphorylation and the underlying oxidation-reduction processes became both Walker's and Hill's predominant concern in these years. To Hill and others, it was evident that Arnon's model was not the solution, since it assumed redox potentials for cytochromes f and b_6 that were more positive than +0.81mV, which did not correspond to the properties that had been observed. [115] However, before I turn to Hill's work once again, I shall look at a third decisive area of development in these years: the investigation of photosynthetic pigments and how these pigments use incident sun solar energy.

4 Energy Migration, Chlorophyll *a* and P700

4.1 Early Observations

Another question that remained to be solved was how the energy of light was "absorbed" by the photosynthetic pigments. In 1936 Gaffron and Kurt Wohl had proposed that a couple of thousand of pigment molecules might "cooperate" in the absorption of light energy and in the reduction of carbon dioxide (based on Emerson and Arnold's 1932 experiments); and even though this sounded like an exciting idea, by as late as the 1950s, Franck, for example, was still strongly opposed to this extravagant notion.[116] The main point of concern was exactly how these "cooperating" light-absorbing molecules could possibly function. Given the speed of photosynthetic carbon reduction, it was impossible to imagine that the carbon

[113] Arnon (1959), p. 16.

[114] Walker (1995), p. 45.

[115] See, e.g., Hill & Bendall (1960), p. 137.

[116] Franck thought that energy transfers of this kind were impossible, pointing to the paper Franck & Teller (1938). The inadequacy of this approach was finally demonstrated in Robinson (1967), while the alternative was first elaborated in Pearlstein (1966), more precisely Pearlstein (1967). See also Chapter III, p. 148.

dioxide moved freely around in the chlorophyll and picked up the required light quanta here and there. Carbon dioxide reduction could only occur at specific sites within the chlorophyll, which Gaffron and Wohl had tentatively called, "reducing centres". The question then was how the absorbed light energy could be transported from the surrounding chlorophyll molecules to these centres.

Around 1940, two explanatory alternatives existed: either the actual particles that carried the energy to the reducing centres might be moving (for example, intermediate radicals), or the energy quanta themselves moved through a structurally coordinated ensemble of chlorophyll molecules in such a way that the energy of these light quanta would finally be captured by the reducing centre (which might or might not be identical with chlorophyll molecules).[117] The latter concept became known as the "optical model of the photosynthetic unit" and was strongly favoured by Wohl as the most probable explanation.[118]

Closely related to how the chlorophyll molecules might be able to interact was the role of the further, so-called auxiliary or accessory pigments, which are present in all photosynthesising plants: the carotenoids, including the xanthophylls (such as fucoxanthin) or, in other organisms, the phycobiliproteins (such as phycoerythrin and phycocyanin). While it seemed beyond any doubt that these pigments also absorbed light energy, no one knew whether or not this energy was used for photosynthesis, and if so, whether it initialised a process different from the one prompted by chlorophyll. These were questions that received considerable attention towards the end of the 1930s – for example, at the University of Wisconsin in Madison, one of the first places in North America where the field of limnology had begun to thrive around 1900.[119] One of the topics of interest here was the theory of chromatic adaptation in submerged aquatic plants, which suggested that the colours of algae enabled them to use optimally the spectrum of the sun's solar radiation penetrating to different sea depths.[120]

In this local context, a study of photosynthesis in the diatom *Nitzschia closterium* (a unicellular marine alga) was undertaken in 1940 by a PhD candidate in plant physiology Herbert J. Dutton. [121] In order to study the relative contribution of the accessory pigment – which in this case was mainly fucoxanthin – the photosynthetic quantum efficiency of the diatom was measured in low light intensities. In particular, Dutton compared the efficiency at wavelengths where the absorption of chlorophyll was most prevalent with the efficiency at wavelengths where mainly

[117] A comprehensive review of this problem was given in Wohl (1940). Note that, at the time, Gaffron and Wohl still thought that: (a) photosynthesis required no more than four light quanta to produce one molecule of oxygen; and (b) that carbon dioxide was also firmly attached to the postulated reducing centres of unknown material identity; see Chapter III, pp. 138ff.

[118] Wohl (1940), p. 47, stressed that Max Delbrück found "that a body with such qualities is physically conceivable. If the intervals between the absorbing centres of the chlorophyll molecules of the body are of atomic dimensions, or in other words, if the chlorophyll molecules are packed flatly one over the other in such a manner that all absorbing centres are in direct contact, then this crystalloid structure will be activated as a whole as soon as a light quantum is absorbed at any point." Note that the celebrated Three-Man-Paper used a rather similar concept to explain the structure and mutation of the "gene"; Wohl (1940), p. 48, explicitly referred to this notion.

[119] Cf., e.g., Beckel (1987).

[120] See also Dutton (1997), p. 175.

[121] The main results were published as Dutton & Manning (1941). The diatom is today known as *Phaeodactylum tricornutum*.

the pigment fucoxanthin was absorbing. Efficiencies were determined in terms of oxygen release. In view of the data, Dutton and his mentor and co-author, the plant physiologist Winston Manning maintained that "it appears necessary to conclude that light absorbed by some or all of the carotenoid pigments in *N. closterium* can be utilized in photosynthesis".[122] These results were elaborated in Dutton, Manning & Duggar (1943), in order to learn whether or not the carotenoids were feeding the absorbed light energy into the chlorophyll pathway. This was determined by using chlorophyll fluorescence at different wavelengths: "If there were no transfer of energy, the yield of chlorophyll fluorescence should vary in proportion to that fraction of the absorbed light which is absorbed by chlorophyll" is how the authors explained the underlying rationale.[123] By contrast, a constant yield at various wavelengths would be strong evidence for the occurrence of the efficient transfer of absorbed energy from other pigments to the chlorophyll – and this in fact was observed: despite the low light absorption of chlorophyll in red light, the fluorescence yield of the diatom *N. closterium* was almost constant over the full range of the visible spectrum:

> From the results [...] it may be concluded that carotenoid-sensitized photosynthesis in *N. closterium* takes place through the transfer of absorbed energy from carotenoid molecules to chlorophyll molecules with subsequent reactions the same as though chlorophyll molecules were the primary absorbers.[124]

Similar conclusions were arrived at by Emerson and Charlton M. Lewis, who, around the same time, had turned to study the role of accessory pigments in the blue- green alga, *Chroococcus*, and found that "light absorbed by phycocyanin is utilized in photosynthesis with an efficiency approximately equal to that of the light absorbed by chlorophyll."[125] In view of these findings, Emerson suggested to his former collaborator William Arnold, who was then working at the Hopkins Marine Station, Pacific Grove, California, to find out "if the energy absorbed by phycocyanin was being transferred to chlorophyll or was the phycocyanin doing photosynthesis."[126] Arnold established that the energy was being transferred to chlorophyll *a* and he went up to Berkeley to talk to the well-known physicist J. Robert Oppenheimer about the problem. The outcome was the idea that this energy transfer was analogous to the "internal conversion" of gamma rays. The suggestion was first presented by Oppenheimer at a meeting of the American Physical Society in 1941, although the concept was only developed in a paper in 1950.[127] Arnold and Oppenheimer confirmed that about 90 per cent of the

[122]Dutton & Manning (1941), p. 525.
[123]Dutton et al. (1943), pp. 308–309
[124]Dutton et al. (1943), p. 312.
[125]Emerson & Lewis (1941*b*), p. 594.
[126]Arnold (1991), p. 77.
[127]See Oppenheimer (1941) and Arnold & Oppenheimer (1950). The general thought was also mentioned in a letter written by Oppenheimer to Wolfgang Pauli dated 16 April 1945, in which he offered a piece "on the analogy between the sensitization of photosynthesis on the one hand, and the internal conversion of gamma rays on the other" as a contribution to an envisaged "Festschrift" for Niels Bohr. See Pauli (1993), p. 269 (Doc. 724).

light energy absorbed by phycocyanin was used in photosynthesis; and they additionally suggested that this energy was transferred to chlorophyll *a* by internal conversion mechanisms, that is, the non-radiative "resonance transfer of energy from one oscillator to another in resonance with it".[128] The study of the effect of auxiliary pigments was later continued by one of Emerson's students, who in Tanada (1951) provided a fine action spectrum of the diatom *Navicula minima* (in which, incidentally, the Red Drop of photosynthetic efficiency was marvellously displayed) and confirmed the high photosynthetic efficiency of fucoxanthin.

4.2 Fluorescence Resonance Transfer

Neither Dutton's nor Emerson and Lewis's results were received with great enthusiasm. It took another three years before E. C. Wassink & Kersten (1946) were able to confirm the findings, and the real excitement about light energy transfer between pigments only got going in the 1950s. The first of these path-breaking studies was undertaken in 1952 by Charles Stacy French and Violet M. K. Young, who examined the energetic side of photosynthesis in the unicellular red alga, *Porphyridium*, with the help of the newly developed "spectrofluorimeter". French and Young were able to demonstrate rather convincingly the transfer of energy between pigments, and suggested that phycocyanin might be an intermediate in the transfer of energy from phycoerythrin to chlorophyll. Furthermore, French and Young speculated that since "phycoerythrin and phycocyanin transfer energy to chlorophyll, it appears probable that chlorophyll plays a specific chemical role in photosynthesis in addition to acting as a light absorber".[129]

In 1952, Louis N. M. Duysens submitted his PhD thesis to the University of Utrecht in the Netherlands. [130] Later that same year, Duysens was invited to present the results of this thesis at the First Gatlinburg Conference on Photosynthesis , which was an extremely efficient way of circulating the findings and of convincing people of the argument in person. By using a newly developed sensitised fluorescence method, Duysens was able to demonstrate the occurrence of far-reaching and highly efficient energy transfers from phycocyanin, phycoerythrin, carotenoids and chlorophyll *b* to chlorophyll *a* in cyanobacteria and algae. According to Duysens's data, all the light energy that was to be used chemically in photosynthesis had to pass through chlorophyll *a*; whatever energy was not transferred this way was lost. Analogously, Duysens found that the energy transfer in purple bacteria had to pass through a specific bacteriochlorophyll, which he named *B*890. Duysens also suggested a potential mechanism for these processes:

> The transfer of electronic excitation energy between pigment molecules as reported above probably takes place through inductive resonance between the excited molecules and the molecules in the ground state, a theory for which [Theodor] Förster has given a quantum-mechanical treatment, with the aid of which the probability of energy transfer can be calculated from experimental

[128] Arnold & Oppenheimer (1950), p. 424. See also Knox (1996) for this episode.
[129] French & Young (1952), p. 889.
[130] Duysen's first results were, in fact,published in Duysens (1951), but see Duysens (1952) for the complete thesis.

> data. Estimations, based on Förster's considerations, are in accordance with, or at least do not contradict, the results recorded above.[131]

The phenomenon in question, which became known as fluorescence resonance energy transfer, had been discovered in 1946 by the German physical chemist Theodor Förster. (Note that the same phenomenon in photosynthesis had been discussed in Oppenheimer (1941), although Förster was unaware of this.) Already in his first publications, Förster had pointed to the possible importance of these energy transfers in photosynthesis, given Gaffron and Wohl's suggestion that a functional photosynthetic unit of chlorophyll molecules might exist.[132] Förster's theory was only widely and internationally received on the publication in 1951 of his monograph; and it was Duysens who productively picked up the notion of induced resonance to explain his comprehensive set of data. The idea was that when a pigment molecule was excited, through the absorption of light energy, it would induce electronic vibrations in a neighbouring molecule, which at the same time would receive an electronic quantum (which was converted, upon transfer, from a higher into a slightly lower energy state). This process was all the more efficient, Duysens summarised in a review of 1956, "(a) the better the overlapping of the fluorescence spectrum of the transferring molecule with the absorption band of the receiving molecule, (b) the smaller the distance between the two molecules, and (c) the greater the fluorescence yield of the transferring molecule and the specific absorption of the receiving molecule".[133] Duysens compared this mechanism to a "bucket brigade", which went from those pigments that were absorbing towards the blue end of the spectrum to those absorbing at longer wavelengths, terminating at a specific type of chlorophyll *a* (or at the bacteriochlorophyll absorbing at 890 nm respectively).

The speculation that there might be "specific" chlorophyll *a* molecules was another important outcome of his study. Duysens reported the confusing and rather paradoxical observation, obtained using the red alga *Porphyra lacineata* (but confirmed in other organisms), that the light quanta absorbed by the phycobilins prompted chlorophyll *a* molecules to a stronger fluorescence intensity than the light quanta absorbed by chlorophyll *a* itself.[134] Duysens maintained:

> From these observations it may be concluded, not only that chlorophyll *a* occurs in these cells in two different modifications, differing in fluorescence yield, but also that energy is transferred from the phycobilins to the highly fluorescent part of chlorophyll *a*, probably with high efficiency, and that consequently energy is not, or only to a slight degree, transferred to the weakly fluorescent chlorophyll *a* molecules.[135]

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[131] Duysens (1951), p. 549.

[132] See, for the original publication of Förster's results and his reflections on photosynthesis, e.g. Förster (1946), Förster (1947) and Förster (1951). On Förster's life and work in general, see, e.g., Porter (1976); Govindjee (2004*a*), p. 19, provides an English translation of some central paragraphs of Förster's 1946 paper.

[133] Duysens (1956), p. 34.

[134] This was in agreement with earlier observations reported in Haxo & Blinks (1950); the authors had used a polarographic method to measure oxygen, which enabled them to measure rapidly photosynthetic action spectra.

[135] Duysens (1951), p. 549.

The two portions of chlorophyll *a* were identified as "active" (high fluorescence yield) as well as "inactive" (low fluorescence yield) in photosynthesis.[136] The light absorbed by phycoerythrin, Duysens suggested, was transferred mainly to chlorophyll *a* of the active type – he presented no convincing argument, however, as to why this should be the case. After having successfully completed his PhD thesis, Duysens pursued these questions of reversible changes of light absorption further. Looking back at this time, Duysens wrote:

> When it had become clear that excitation energy was transferred to photosynthesis via (bacterio)chlorophyll, I began thinking about a method for studying the photochemical events following the excitation of these chlorophyllous pigments. I reasoned that if chlorophyll participated directly in the photochemical reaction, its absorption spectrum would presumably change, like that of other pigments upon oxidation or reduction. The possible absorption changes, occurring in the photosynthesizing cells upon illumination, should be small, since otherwise they would have been discovered already by the naked eye, which is rather sensitive to color changes.[137]

If the energy were transferred to a certain fraction of the chlorophyll or bacteriochlorophyll, which presumably was only present in low concentrations, this hypothetical pigment P could then be assumed to be photochemically active and change its absorption spectrum upon illumination. Based on this assumption, Duysens set out to study the absorption changes of the different molecules present in the chloroplast. In a letter to the editor of *Nature*, published in April 1954, he reported, with reference to data recently obtained, that "in living bacteria in the absence of oxygen and in the presence of substrate a cytochrome pigment is oxidized by illumination and is reduced in the dark". This was concluded from the observation that in purple bacteria the absorbance change upon illumination "is similar to the spectrum obtained by subtracting the spectrum of reduced cytochrome *c* from that of oxidized cytochrome *c*. This indicates that a cytochrome pigment is oxidized by a light reaction and is reduced by a dark reaction, as indicated by the restoration of the original absorption in the dark". Duysens thus felt entitled to propose that "our experiments indicate that the photosynthetic oxidation of the substrate in *Rhodospirillum rubrum* is mediated by a cytochrome pigment".[138] (Note that, at the time, with the exception of Hill's laboratory at the University of Cambridge, very few scientists believed that cytochromes played a role in photosynthesis.)

The question that naturally followed was whether the same was true of green plants: that here, too, the oxidation of the reductant – water – was mediated by a cytochrome. This would imply that in higher plants and algae light-induced changes of the cytochrome's oxidation state also occurred. At the time, Duysens had gone to spend a period of research in French's laboratory at the Carnegie Institution's Department of Plant Biology, near Stanford. When Duysens told French about his recent research interests, he was told that French himself, together with Britton Chance, had been looking in vain for light-induced absorption changes

[136] Note that these "inactive" portions of chlorophyll *a* were later identified as part of photosystem I, in which TPN, more precisely NADP, is reduced.

[137] Duysens (1989), p. 67.

[138] Duysens (1954*b*), p. 692.

in *Chlorella*; however, they had used Chance's apparatus, and did not then have the advantages of an absorption difference spectrophotometer. Duysens thought that he could put together a home-made instrument of that type, and French encouraged him to try.[139] The results of this study were promising, yet unfortunately less clear-cut than in the case of bacteria:

> We found that *Chlorella*, too, showed a change in the absorption spectrum upon irradiation, which was reversed upon darkening. [...] As a whole, this spectrum of *Chlorella* cannot be identified with the difference between the absorption spectra of oxidized and reduced cytochrome *c* or *f*; it may, however, be the sum of two difference spectra: one belonging to a cytochrome, with the peak at 420 mμ, and one belonging to another pigment, with peaks at 515 and 478 mμ. If this is the correct interpretation, then the absorption drop at 420 mμ can be considered as revealing the oxidation of a cytochrome upon irradiation of *Chlorella* and its reduction in the dark.[140]

Duysens added that this "would be in accordance with the suggestion of Hill and co-workers that cytochrome *f* which they found in the chloroplasts of green plants and in *Chlorella*, functions in photosynthesis as an oxidation-reduction catalyst".[141] Duysens was keen to catch up in respect to this point. In December of the same year, Duysens wrote to Hill:

> My clearest cytochrome spectrum of algae is that of *Porphyridium cruentum*. The difference spectrum has a small peak at 555 mμ, which is the same wavelength as you found for cytochrome *f*. The locations of the blue peaks of the difference spectra of *Porphyridium* and *f* differ somewhat, but the difference spectrum of *f* could not very precisely be determined by me from your published spectra. It would be necessary to have the precise difference spectrum (preferably in tabulated form for every 5-10 mμ) of the cytochrome *f* of *Porphyridium cruentum*, in order to identify with some degree of certainty the *Porphyr.* cytochrome. I would be very happy, if you decided to extract the cytochrome *f* from *P[orphyridium]* which may well contain *f* in larger quantities than *Chlorella*.
>
> In *Chlorella*, there is only a small peak in the difference spectrum, due to a cytochrome which is superimposed by a much "stronger" spectrum. Under favourable conditions, it is possible to find a very small dip near 555 mμ. There is so far no evidence for a cytochrome that is very much different from cytochrome *f* or *c*, in the difference spectrum of *Porphyridium*. This does not exclude, of course a participation of such a cytochrome in photosynthesis. [142]

The problem was that, in *Chlorella*, in addition to the peak of the difference spectrum associated with the changes of the oxidation state of a cytochrome at 420

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[139] See Duysens (1989), p. 68.

[140] Duysens (1954*a*), p. 353. The historical "millimicron" (mμ) unit equals the modern nanometer.

[141] Duysens (1954*a*), pp. 353–354. If he hadn't observed the changes in the cytochrome's absorbance, Duysens would not have thought that the mere presence of a substance in chloroplast could entitle one to make any assumption about its function: "Photosynthetic phosphorylation had not yet been discovered, and it was quite possible that other processes, such as respiration, occurred in the chloroplasts in addition to oxygen evolution and the light reactions." See Duysens (1989), p. 69.

[142] Cambridge University Library, Ms. Add. 9267/**XXX**. Duysens to Hill, Dec. 19, 1954. Read "nm" for "mμ".

nm, there was a much higher peak at 520 nm and another at 480 nm, both of which could not possibly be associated with cytochromes. By contrast, the data gathered using the red alga *Porphyridium* were in good agreement with the assumption that cytochrome *f* was being reduced and oxidised, and the curious peaks at 520 nm and 480 nm were absent this finding was published as Duysens (1955). However, in view of the difficulties he had had with the algae, which Hill had also been unable to resolve, Duysens went back to study the more simple systems of bacteria. (Although, of course, Duysens did not know it at the time, the action spectra of photosynthesis can only provide useful information quantitatively when one photoreaction is involved. Therefore, it is not at all surprising that scientists working in 1955 obtained confusing or even paradoxical results from oxygen-producing organisms, in which photosynthesis is driven by two photoreactions, although this was then unknown. Reverting to simple systems was probably the wisest course of action to take at the time.)

Duysens again improved the instrumental set-up and devised an extremely sensitive differential spectrophotometer that was able to detect even minute changes of optical absorbance.[143] In 1956, Duysens suggested that his findings were compatible with the effect of oxidation-reduction processes in which bacteriochlorophyll and cytochromes were involved.[144] His proposal can be expressed in the form of an equation as:

$$\text{BChl} + \text{Light} \rightarrow \text{BChl}^*$$

$$\text{BChl}^* + \text{X} \rightarrow \text{BChl}^{ox} + \text{XH (photosynthetic reductant)}$$

$$\text{BChl}^{ox} + \text{cyt}^{red} + \text{proton(s)} \rightarrow \text{BChl} + \text{cyt}^{ox}$$

Thus, the process was considered to be the following: upon illumination, excited bacteriochlorophyll (BChl*) was formed and photooxidised, while simultaneously an unknown substance, X, would be reduced to XH, which acted as the photosynthetic reductant. The oxidised bacteriochlorophyll (BChl^{ox}) might be replenished again by oxidising one or more reduced cytochrome compounds. A fraction of the oxidised cytochrome, Duysens thought, might return to its reduced state by oxidising the XH, thereby releasing the energy that was required to generate ATP. Yet, Duysens et al. felt compelled to add: "It should, however, be pointed out that this is not the only possible interpretation."[145] A reconstruction of this idea is given in figure VI.8.

At the time, Duysens believed that something similar might apply to photosynthesis in higher organisms. The difference between the free energy of XH and TPN was sufficient for the phosphorylation of one ADP molecule per two reducing equivalents to take place, and could explain non-cyclic photophosphorylation coupled to the production of TPNH and oxygen. It was not at all necessary to assume, as it was later done, that there existed two sequential photochemical reactions. Duysens even added that such a scheme would have presumed a minimum quantum

[143] The first findings with this instrument were reported in Duysens, Huiskamp, Vos & van der Hart (1956), p. 188.

[144] See Duysens et al. (1956), p. 190.

[145] Duysens et al. (1956), p. 190. The suggestion was repeated and slightly elaborated in Duysens (1957).

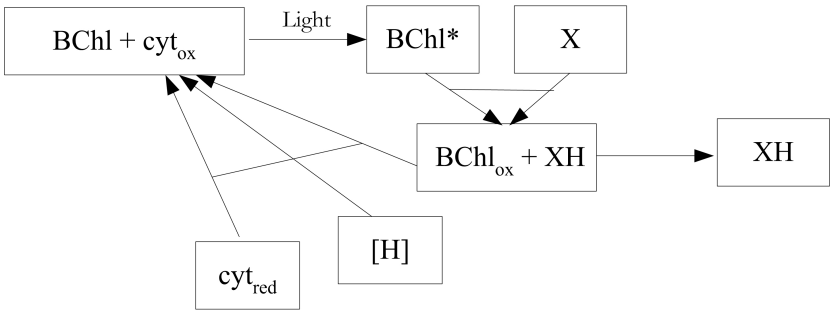

Figure VI.8: The cyclic process of reversible oxidation of cytochromes and chlorophylls upon illumination. (XH = photosynthetic reductant.)

requirement of eight, if it was assumed (as Duysens had proposed in his thesis) that one quantum was needed to produce one reducing equivalent. At the time, however, Duysens thought that the most reliable quantum requirement determination had been carried out by Frederick S. Brackett in 1953, who had measured a requirement of 6.1± 0.6: "In view of the evidence available at that time, Hill and Bendall's later proposal of two reactions in series was an unlikely hypothesis."[146] Duysens still believed in 1958 that "there was no convincing indication that two major photosynthetic reactions might be cooperating in oxygenic photosynthesis", but everything changed the following year.[147]

It was in 1957 that Seymour Steven Brody and Rabinowitch, working at the University of Illinois at Urbana–Champaign (US), were able to provide evidence for the first time of the excitation energy transfer from their kinetic studies.[148] They were able to obtain their evidence by means of ultrafast fluorescence spectroscopy experiments, which were able to trace the excitation lifetime of photosynthetic pigments. Note that these times were in the order of nanoseconds, that is, 10^{-9} seconds; and it was no trivial exercise to provide such a short light pulse in 1957. Ordinary flash lamps were far too slow, so that Brody and Rabinowitch eventually used a small hydrogen lamp for this purpose. The drawback of the latter was the relatively low intensity of this lamp, so that all the components of the experimental set-up – lamp, coloured glass filters, sample and photodetector – had to be assembled closely together.

The fluorescence signal induced by the flash was detected by a photomultiplier, which was applied directly to the plates of an oscilloscope, and the display was then photographed. "The fluorescence lifetime T of several pigments was determined *in vitro* in this way with a precision of ± 7 per cent, and – for the first time – also

[146] Duysens (1989), p. 71. For the measured value, see: Brackett (1953).
[147] Duysens (1989), p. 71.
[148] See Brody & Rabinowitch (1957) for the original publication. Brody (2002) provides a historical and partly autobiographical mini-review of fluorescence lifetime studies and energy transfer in photosynthesis.

in vivo, with a precision of ± 20 per cent," Brody and Rabinowitch explained.[149] However, in order to achieve this degree of precision, which was excellent for the time, a considerable number of practical difficulties had to be resolved. One of these concerned the fact that the only high-speed oscilloscope available on the Urbana campus was in the university's cyclotron laboratory, which was mainly used for other purposes. "The only times I could reserve the use of the oscilloscope was from midnight until about 8 o'clock in the morning. So for almost half a year I worked the midnight shift," Brody recalled. The positive aspect of doing the "midnight shift" was that Brody had few distractions and so was able to get a lot of work done.[150] Brody and Rabinowitch found that the measured values for excited chlorophyll molecules *in vivo* differed greatly from the previously calculated values (while the data *in vitro* were in good agreement with the calculations). The authors suggested:

> One possible interpretation of this discrepancy is to assume two forms of chlorophyll *in vivo* (a hypothesis for which some spectroscopic evidence has been obtained by other investigators); the fluorescent form must then account for about one fourth of the total, and the non-fluorescent form for about three-fourths of the total.[151]

This would have nicely confirmed Duysens's findings using red algae. Brody and Rabinowitch admitted, however, that the discrepancy could be attributed to the special conditions of the experiment and to possible differences in the physiological state of the algae. Equally important was their finding that it proved possible to measure the times required for the transfer of excitation energy. In retrospect, Brody described the results as follows:

> The phycoerythrin was irradiated with a nanosecond burst of green light. The excitation energy absorbed by phycoerythrin is transferred to phycocyanin and subsequently to chlorophyll. Some of the excitation energy transferred to chlorophyll is emitted as fluorescence. The time between the nanosecond burst of green light and the appearance of the red fluorescence from chlorophyll is the time required to transfer excitation energy in the red alga. [...] The measured time for energy transfer is 0.5ns.[152]

Thus, by 1958 there could be little doubt that several different pigments contributed their excitation energy to photosynthesis, while chlorophyll *a* was the only pigment directly responsible for making the excitation energy available for the enzymatic steps in photosynthesis. It not only absorbed sunlight itself; it was also the final step in a chain of highly efficient transfers of excitation energy from those pigments with absorption bands at shorter wavelengths to those with bands at longer wavelengths.[153] Whether or not there were, in fac,t different types of

[149]Brody & Rabinowitch (1957), p. 555. Note that the lifetime of chlorophyll *a* fluorescence *in vivo* was also measured, independently and using other methods, by Dmetrievsky, Ermolaev & N. (1957).

[150]See Brody (1995), pp. 68–69.

[151]Brody & Rabinowitch (1957), p. 555.

[152]Brody (2002), p. 129, describing the results of Brody & Rabinowitch (1957).

[153]See, e.g., Emerson & Chalmers (1958), p. 15, for a succinct summary of the situation up to then. See also Emerson & Rabinowitch (1960), p. 477.

chlorophyll *a*, only one of which was photosynthetically active, was a question that awaited further clarification.

4.3 Special Pigments and the Antagonistic Light Effect

While in 1956 Duysens had found certain unusual and particularly important molecules of bacteriochlorophyll, which he had dubbed "P890" and "P870", Bessel Kok, then working in Wageningen, The Netherlands, discovered that same year a similar pigment in higher plants, algae and cyanobacteria. In studies very similar to the ones undertaken by Duysens, Kok had observed that "all plants containing chlorophyll *a* showed, after irradiation with a strong light flash, a temporary decrease in absorption (lifetime in the order of 0.01 sec) in the spectral area between 620 mμ and 720 mμ".[154] Already then, Kok interpreted these changes as indicating "the photochemical transformation of a pigment that is different from chlorophyll *a* in its normal status and that occurs universally in the plant kingdom".[155] In 1957, Kok confirmed this finding of a short-lived, light-induced absorbance decrease in several photosynthetic organisms, which had its highest wavelength at 700 nm; whereupon he labelled the pigment in question "P700".[156]

Kok (1959) presented a much improved apparatus, which was applied to the study of absorbance changes in the cyanobacterium *Anacystis nidulans*. Although the data were still complex (and confusing), they seemed to indicate "that far-red light yields a decrease of background absorption whereas red or white light has the reverse effect".[157] Kok drew attention to the fact that an interpretation of this finding could be provided in view of the discovery of enhancement effects by Emerson and his co-workers: they had found that, at low light intensities, the severe drop in photosynthetic efficiency for wavelengths beyond 680nm could be compensated for by the simultaneous illumination by red light of shorter wavelengths (see Chapter IV, Section 8.1).[158]

Kok considered the following:

> In the light of Emerson's findings we can at least tentatively try to explain some of the effects we have observed. The location of the absorption band of the 700 mμ pigment – even if present in only a very small concentration – makes it ideally suited to effectively trap all the light which is absorbed by chlorophyll *a* itself, or transferred to it by accessory pigments (and which thus is transformed into "far-red" light). Suppose the 700 pigment is indeed the final light sink in photosynthesis, and its excitation by surrounding chlorophyll *a* molecules leads to a conversion, which entails disappearance of its absorption. Then, from there on, the neighboring chlorophyll *a* molecules – say those comprising a "unit" – are deprived of their outlet until the 700 pigment is restored. This restoration, however, also requires light, but now only red and not far-red is active. Obviously, sensitization by chlorophyll *a* (which yields only 700 mμ fluorescence) cannot restore the bleached 700 pigment and photosynthesis cannot proceed effectively in far-red light alone. As was described above, irradiation with red light restores the 700 mμ pig-

[154] Kok (1956), p. 399. Read "nm" for "mμ".
[155] Kok (1956), p. 401.
[156] Kok (1957).
[157] Kok (1959), p. 190.
[158] The main papers are Emerson et al. (1957) and Emerson & Chalmers (1958).

> ment and therefore sustains the conversion of irradiation transferred to it via chlorophyll *a*.[159]

Evidently, Kok was already then playing with the idea of two different photochemical reactions requiring light of different wavelengths, which would act in sequence upon P700. Yet, Kok hesitated to push these speculations further, and explicitly mentioned that he would not have published the results and their preliminary interpretation "if it were not to draw attention to a new road towards a better understanding of the most fundamental aspects of photosynthesis, opened up by the eminent scientist to whose memory this article is dedicated".[160] This alluded to Emerson's finding of the Enhancement Effect (which already then had made it very probable that two different pigments with different absorption properties initiated two different photochemical reactions) and to his untimely death in an aircrash, as Kok's paper was printed in a memorial issue of *Plant Physiology*, dedicated to the memory of Emerson. Kok did continue his thinking on two different photo systems, the results of which will be presented in Section 5.4 of this chapter.

5 A Solution that Was in the Air

To sum up, by 1959 it was known that chloroplasts – at least *in vitro* – were able to utilise light energy to form, on the one hand, TPNH (that is, reducing equivalents), upon which a stoichiometric amount of molecular oxygen was released,and, on the other hand, ATP, provided that the chloroplasts were supplied with ADP and inorganic phosphate. The four hydrogen equivalents required to reduce the necessary number of TPNs were, most probably, taken from water molecules. For each molecule of oxygen thus released, approximately two ATPs were found to be formed. Furthermore, it was suspected that electron transport chains with a slow and stepwise energy release were involved in the formation of ATP, and that cytochromes (and the factor that was later identified as ferredoxin) were possibly part of this chain – notably the cytochromes discovered at Cambridge to be specifically present in green plant tissues: cytochromes f and b_6. Support for the involvement of cytochrome f was provided by Duysens's finding that it was reversibly oxidised in the light. In addition, it was also suspected that there might be different types of chlorophyll *a*, which presumably were relevant to the course of the light reaction; there was Kok's mysterious P700, which reacted antagonistically to light of different wavelengths. And, finally, Emerson's finding of an Enhancement Effect were already being discussed in terms of a "short wavelength" and a "long wavelength" pigment system that somehow acted cooperatively to achieve photosynthesis in plant cells.

These different strands of evidence were drawn together by a number of research groups, which more or less simultaneously came to similar conclusions, albeit from different starting points. Around 1960, the problem was to find as detailed a model as possible, which was able to accommodate, if not *explain*, most of the pertinent data and, at the same time, adhere to theoretical preconditions, such as the known

[159] Kok (1959), pp. 191–192. Read "nm" for "mμ".
[160] Kok (1959), p. 192.

redox potentials of certain molecules. Hill and Fay Bendall were the first to publish, in 1960, how, from a thermodynamical point of view, the cytochromes could be arranged in a redox chain so as to provide enough energy to form ATP. Kok elaborated his aforementioned suggestion, based on his finding of P700; although neither Hill nor Kok recognised the role of different pigments for the different light reactions, which had been discussed since the finding of the Emerson Enhancement Effect. This insight provided the starting point, however, for the research carried out by the groups working around Duysens and the German biophysicist Horst T. Witt; and it guided further studies of the different types of chlorophyll carried out at Urbana by Rabinowitch and Govindjee. In these studies, as well as in the work done by the German chemist Hans Kautsky, it also emerged that the course of chlorophyll *a* fluorescence was best explained by assuming that there were two different photochemical reactions. All these contributions complemented each other, and all were decisive in endorsing the two photoreactions, two pigment systems model, which in outline still holds today.[161]

5.1 Far-sighted Ideas at Urbana

It needs to be emphasised that what was achieved around 1960 was not the discovery of the possible existence of two different photochemical reactions. This idea, as a viable explanatory approach, had been around since Rabinowitch's monograph of 1945 at the latest – indeed, the notion that there was more than one photochemical process had been a common assumption since the early 1930s. It was mentioned in Chapter III that various actors, including Arthur Stoll, Richard Willstätter and Franck, believed that several photochemical steps were needed to achieve carbon dioxide reduction (which, at the time, was thought to be the light reaction); while in Franck & Herzfeld (1941) it was argued that there were eight photochemical steps of fundamentally the same nature, each requiring the energy of one light quantum. These schemes lost their attraction when it emerged that the light reaction was concerned with the "splitting" of water and the dark reaction effected the reduction of carbon dioxide; yet, it remained to be explained why so many light quanta seemed to be necessary necessary to complete the full reaction series of photosynthesis.

When Rabinowitch discussed the various models of the primary photochemical process in the first volume of his photosynthesis monograph in 1945, he included the mechanism drafted in Franck & Herzfeld (1941) in his list of promising models. Rabinowitch believed that two alternatives might explain why photosynthesis seemed to require eight light quanta (which he already then assumed was the accurate number; see, however, Chapter IV): either one could "activate the same four hydrogen atoms *photochemically* twice in succession" or one could "double the number of identical primary photochemical processes".[162] Rabinowitch started with the first of these alternatives, which, he believed, had to involve photo-oxidations (in which hydrogen atoms were taken away from the water) and photo-reductions (in which the same hydrogen atoms were transferred to carbon dioxide or an

[161] The story of how it was established that there are two photosystems has been told repeatedly, mainly by the actors themselves or close collaborators; see, e.g., Duysens (1989), Witt (1991) and Amesz (1998). Some previously neglected aspects can be found in Govindjee (2006).

[162] Rabinowitch (1945), p. 161.

intermediate acceptor). In order to clarify this option, Rabinowitch designed a scheme in which photosynthesis was framed as a series of redox reactions between three intermediary catalysts: X, Y and Z (see figure VI.9).[163] The first light reaction effected the transfer of hydrogen from HZ to Y, upon which Z reacted with H_2O to evolve oxygen. The second light reaction transferred hydrogen from HY to X, upon which HX reduced carbon dioxide to carbohydrates. (Rabinowitch refrained from discussing the nature of the intermediates X, Y or Z, although he thought it probable that at least the central intermediate, Y, which participated in both photochemical reactions, was chlorophyll.) This possibility was then contrasted with the aforementioned suggestion cited in Franck & Herzfeld (1941), in which eight quanta were used up by eight identical photochemical processes.[164] After weighing up the arguments for each of these alternatives, Rabinowitch concluded that, at the time, the second approach (involving eight identical primary processes) was to be preferred. He emphasised, however, that the former option, of two sets of different primary processes, would become first choice "if the existence of two interconvertible green modifications of chlorophyll – one a photo-oxidant and one a photo-reductant – would be definitely confirmed by experiments *in vitro*".[165]

The issue had to be re-considered in the 1950s for a number of reasons. Rabinowitch himself again brought forward the possibility of two different light reactions in the second volume (1956) of his photosynthesis monograph, when he tried to find an explanation for Duysens's experiments of 1954, as well as for similar findings of the Swedish plant physiologist Henrik Lundegårdh.[166] Having discussed at length the experiments and their outcome (that is, the observation of light-induced cytochrome *f* oxidation in photosynthesising cells), Rabinowitch considered two alternative interpretations: either the findings could be taken "as evidence of direct participation of these compounds in the photochemical hydrogen transfer from water to carbon dioxide (or, rather, to an organic compound into which CO_2 had been incorporated, such as PGA)"; or, as suggested in Duysens et al. (1956), they could be taken "as evidence of their participation in oxidative processes (back reactions), coupled with the reduction process".[167] Rabinowitch's preference was clear:

> The first hypothesis [...] suggests photochemical transfer of electrons *from* reduced cytochrome to the organic acceptor (perhaps via DPN or TPN). *The transfer of hydrogen (or electrons) from H_2O to the oxidized cytochrome would then require another photochemical reaction.* To account for the observed shift, the relative probability of the two photochemical reactions would have to be such as to establish a photostationary state with most of the

[163] Rabinowitch (1945), p. 162, scheme 7.V.

[164] Rabinowitch (1945), pp. 163–165, scheme 7.V A.

[165] Rabinowitch (1945), p. 168. Both Govindjee and Duysens have drawn attention to this passage of Rabinowitch's book; see, e.g., ; Govindjee (1995), p. 139, Govindjee (2006), p. 154, and Duysens (1989). Note that Rabinowitch himself pointed to his earlier thoughts in Rabinowitch (1963) (p. 113) and emphasised that by then the evidence strongly supported the first alternative of "two consecutive sets of four transfers [of hydrogen/electrons] each".

[166] The relevant paper is Lundegårdh (1954). On Lundegårdh's life and work, see, e.g., Larkum (2003).

[167] Rabinowitch (1956), p. 1862. See p. 362 of this Chapter for more details on Duysen's own interpretation.

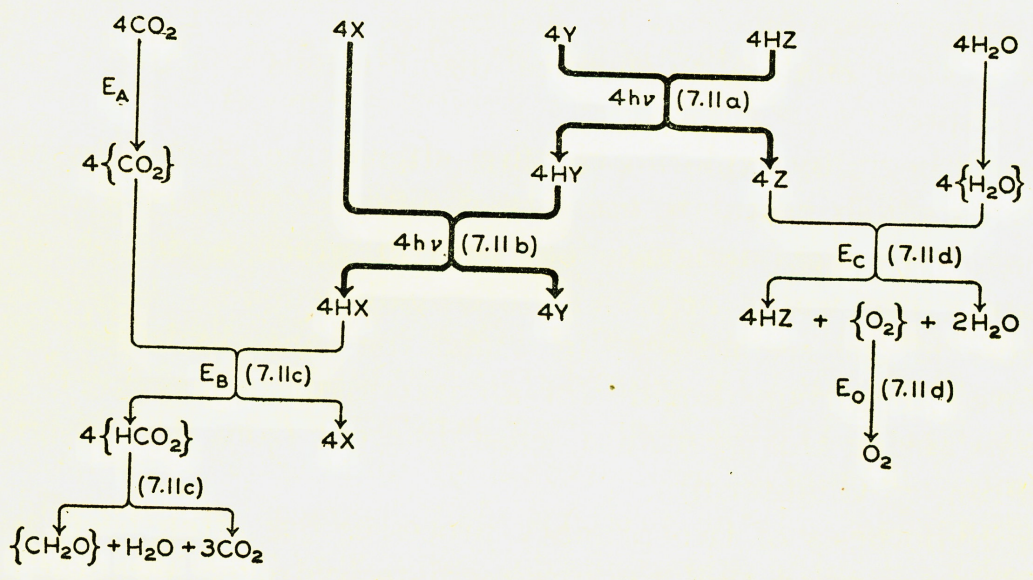

Figure VI.9: Reproduced from Rabinowitch (1945), p. 162: "Photosynthesis with oxidation-reduction reactions between three intermediary catalysts as the two primary photochemical processes. (The central catalyst, which participates in both photochemical reactions, may be chlorophyll."

> cytochrome in the oxidized state. The quantum requirement of the hydrogen transfer reaction as a whole would be (at least) 8, since two quanta will be needed to transfer each of the four required H atoms (or electrons), *first from water to the cytochrome, and then from the cytochrome to the final acceptor.*[168]

Thus, already in 1956, a fairly precise suggestion of the photosynthetic electron transport chain, with cytochrome as an intermediate, was publicly presented – if only as one of two alternative interpretations of Duysens's data (the importance of which was realised by most photosynthesis researchers only much later). Govindjee, who after Emerson's death completed his PhD thesis under Rabinowitch and later became Emerson's successor at Urbana, still remembers that in the second half of the 1950s every one in the Urbana laboratory was fully aware that photosynthesis involved two photochemical reactions, most probably via cytochrome *f*, so that they were astonished to observe the stir that was caused by the publication of Hill and Bendall along these lines. Had not Rabinowitch published all this four years earlier?[169] I shall now turn to the Hill–Bendall contribution.

[168] Rabinowitch (1956), p. 1862. Govindjee's italic. This passage was discovered independently (and highly appreciatively) in 1956 by two of Rabinowitch's closest associates, Govindjee and Duysens. It has been acknowledged in many talks, in particular given by Govindjee, and has been quoted in written form, e.g., in Duysens (1989), p. 74, and in Govindjee (2006), p. 154.

[169] Personal communication, Govindjee to the author, in September 2005. It seems that outside Urbana hardly anybody at the time had read Rabinowitch's volumes very carefully, so that many of his contemporaries were unaware of his insightful suggestions.

5.2 The Thermodynamical Approach

The main focus of Hill's work in the 1950s was solving how cytochromes f and b_6 functioned in a photosynthetic electron transport chain. In addition to the general suspicion that respiration and photosynthesis might operate along roughly similar lines, Hill thought that the molecular ratio of cytochromes f and b_6 to chlorophyll, which were found to be about 1:4000, were highly suggestive, since these were, by analogy with the relationship between cytochrome c concentration and respiration rate, "of the right order to account for the rates of photosynthesis in terms of hydrogen transport".[170] A proposal was eventually published in the paper Hill & Bendall (1960), entitled "Function of the two cytochrome components in chloroplasts: A working hypothesis".[171] Hill addressed the problem from the point of view of thermodynamics; and while he did not cite Rabinowitch's monograph, it is very likely that Hill was strongly influenced by how the subject was treated therein.[172]

Hill and Bendall began their paper by summarising the generally accepted body of knowledge on the issues under consideration, notably the relationship between the transfer of hydrogen from water to the hydrogen acceptor and the formation of ATP from ADP and P_i in the chloroplast. The hydrogen transfer, the authors wrote, could be represented by the following set of equations:

$$H_2O + X + Y \longrightarrow XH + YOH \tag{VI.3}$$

$$XH \longrightarrow X + (H) \tag{VI.4}$$

$$YOH \longrightarrow Y + \frac{1}{2}H_2O + \frac{1}{4}O_2 \tag{VI.5}$$

This could be condensed to the following summary equation:

$$\frac{1}{2}H_2O \longrightarrow (H) + \frac{1}{4}O_2 \tag{VI.6}$$

The same process, Hill and Bendall suggested, could also be represented in the form of a diagram (see fig. VI.10). The oxidised and reduced parts of the water would be separated from each other by the reaction with the primary acceptors, provisionally called X and Y. The [H] would then be transferred to an appropriate secondary acceptor (eventually TPN), while the [OH] moieties would react with each other and release molecular oxygen and water. Thus far, Hill and Bendall were in line with what was generally assumed to be the case.

Given the redox potentials of the hypothetical participants, notably cytochromes f and b_6 as intermediate reactants, Hill and Bendall then argued that, at first

[170] Hill (1965), pp. 133–134.

[171] Very similar ideas are formulated in Hill & Bonner (1961), although it is unclear how much of this? was already presented at the conference or elaborated for the proceedings. The paper Hill & Bendall (1960) is cited in the list of references.

[172] See, e.g., the treatment of photosynthesis in Rabinowitch (1945), Chapter VII (pp. 150–171). I am grateful to Govindjee for pointing out this connection to me.

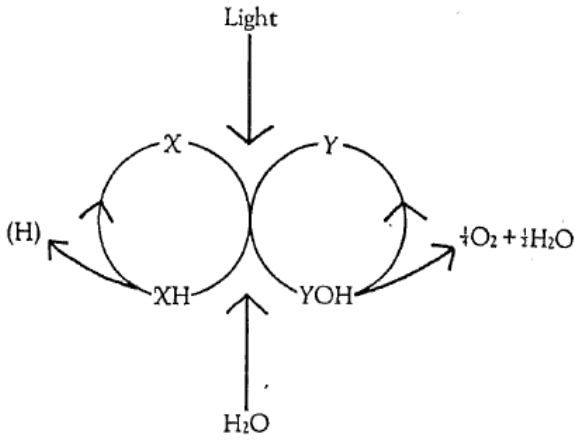

Fig. 2. *X* and *Y* are the hypothetical first acceptors of the products of the photolysis of water, forming respectively the reduced product *X*H and the oxidized product *Y*OH

Figure VI.10: The photolysis of water and the hypothetical first products taken from Hill & Bendall (1960), p. 136.

glance, the electron transport chain seemed to require three separate light-driven reactions on the way from YOH to XH (left-hand-side diagram of fig. VI.11). However, as this was not in accordance with experimental evidence, Hill and Bendall suggested a system of two light-driven reactions, with a spontaneous "back reaction" in between (right-hand-side diagram of fig. VI.11):

> The postulation of two light-driven steps, rather than three would be in better accord with present experimental results. In this case oxidized cytochrome b_6 would have to be reduced by Y to give YOH and cytochrome f would have to be oxidized by X to give XH. The reaction between cytochromes f and b_6 would then be a thermochemical process and quite analogous with a hydrogen transfer step characteristic of the mitochondrion. [173]

Under "normal" conditions in the cell, cytochrome f would tend to be in its reduced form and cytochrome b_6 in its oxidised form. When the cell was illuminated, Hill and Bendall argued, the redox states were reversed: the unknown primary acceptor, X, acted as an oxidising agent, turning cytochrome f into its oxidised state; while at the same time, the unknown reducing agent, Y, turned cytochrome b_6 into its reduced state. Under normal circumstances the two cytochromes would spontaneously and very quickly react with each other and return to their favoured oxidation state, while the remainder of the energy release might be used to synthesise ATP. In a comparison with their first figure (labelled as Figure 2), this model was also represented by the authors in fig. VI.12. Hill and Bendall admitted that, the nature of X and Y was still obscure, and that the whole suggestion had to be taken as a rough hypothesis.[174] They also rather openly

[173] Hill & Bendall (1960), p. 137.
[174] All quotes: Hill & Bendall (1960), p. 137.

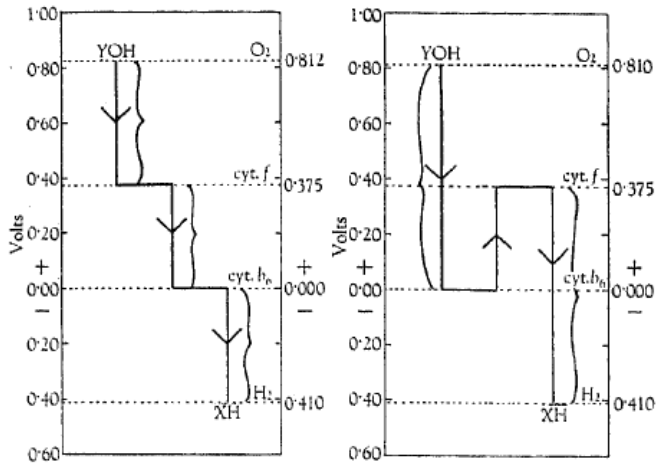

Figure VI.11: The hypothetical electron transport chain in terms of oxidation-reduction potential. On the left, a potential model with three light-driven steps, which was abandoned; on the right, the favoured model with two light-driven steps, connected by a thermochemical reaction along the gradient between the two cytochromes; taken from Hill & Bendall (1960), p. 137. (The downward arrows symbolise electron transport *against* the thermochemical gradient.)

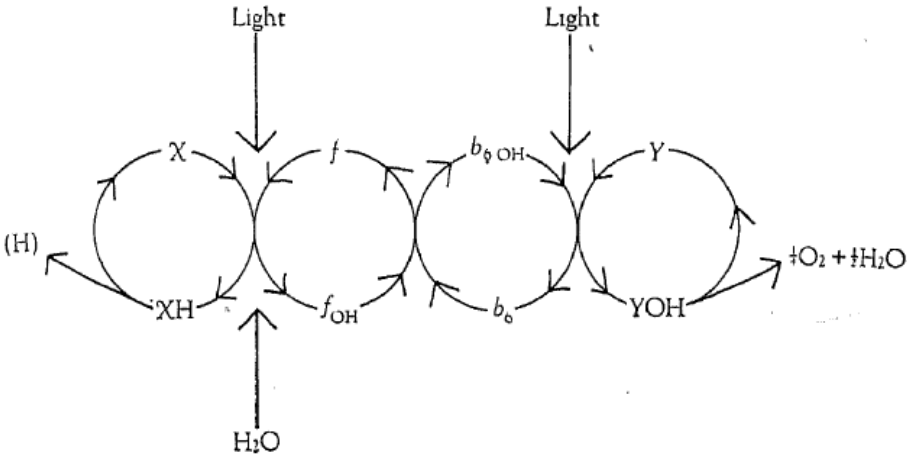

Fig. 5. *X*, *Y*, *XH* and *YOH* as in Fig. 2. *f* and b_6 represent ferrous and f_{OH} and b_{6OH} ferric or oxidized cytochromes

Figure VI.12: The two photoreactions model of photosynthesis taken from Hill & Bendall (1960), p. 137.

acknowledged their own lack of empirical support.[175] However, the authors also underlined that the model had the definite advantage, in contrast to the one put forward by Arnon and his collaborators, for example, that it was in accordance with the known redox potentials of all the components involved. Whereas Arnon's group had assumed a role for the cytochromes at one or other end of the photochemical sequence, Hill and Bendall envisaged the cytochromes in an intermediate position, between the two photoreactions, which was much more convincing.

The proposal provided a surprising solution to the problem of where the energy to produce ATP came from (namely, from the energy drop between the two cytochromes);[176] it preserved the far-reaching correlation between photosynthesis and respiration; and it was in agreement with the curious observation that in the light the two cytochromes tended to be in the energetically disadvantageous state, that is, when illuminated, cytochrome f was found in its oxidised mode, notwithstanding its highly positive redox potential, while cytochrome b_6 was found to be reduced. It is unclear, however, why Hill did not refer to the actual finding of this phenomenon in red algae, by Duysens (1954*b*) and Duysens (1954*a*) (both of which Hill knew, as can be taken from his correspondence), but merely expressed the hope that it might be possible to study these effects in purple bacteria. Furthermore, Hill and Bendall did not refer to the obvious relationship of this suggestion to the Emerson Enhancement Effect, with which Hill was clearly familiar: the conclusion that two different photochemical reactions were required in photosynthesis, which in Hill and Bendall's paper was featured as the result of purely thermodynamic considerations, had been arrived at on the basis of experiments carried out three years earlier. At least in this paper, Hill appeared not to be interested in tying down thermodynamics to experiments and pigment systems; he also displayed a surprising indifference to giving due credit to the earlier achievements of his colleagues.

5.3 Spectroscopy and Two Photosystems

In fact, a number of these colleagues arrived at very similar conclusions around the same time. Duysens became interested in the strange effect of different wavelengths on photosynthetic transient states in the mid-1950s (that is, around the same time as Emerson; see Chapter IV, p. 237). Duysens later recalled that at the second Gatlinburg conference in 1955 , Lawrence Blinks had presented data which indicated that "in red algae, illuminated alternately with red and green light for a few minutes, qualitatively different photosynthetic transients occurred, which were not caused by differences in intensity".[177] Duysens thought that these phenomena

[175] "The only evidence for the above hypothesis, in higher plants, is the observation that illumination of pale yellow-green leaves causes an oxidation of [cytochrome] f and the reduction of b_6. As yet, this sequence of reactions has not been observed, in our laboratories, with normal green leaves or chloroplasts. [...] It is our hope that future investigations will be able to elucidate more fully the role of cytochromes in the photosynthetic process of green plants." Hill & Bonner (1961), p. 434.

[176] It transpired, however, that the role of cytochrome b_6 differed from the one that Hill and Bendall had envisaged; it is involved in the process of cyclic electron transport.

[177] See Duysens (1989), p. 71. The data were published two years later in Blinks (1957); see also Blinks (1960*a*), Blinks (1960*c*) and Blinks (1960*b*). Like Emerson, Blinks himself proposed that these effects were caused by the side reactions of respiration; this was proven to be erroneous by

– today known as the "chromatic transients" of oxygen exchange – were most probably caused by the overlapping effects of two or more photochemical reactions with different action spectra. (Duysens did not assume, at the time, that all these reactions were actually part of photosynthesis.) He received more inspiration from the paper Emerson et al. (1957), in which the Enhancement Effect was presented. Duysens was keenly interested in this phenomenon, although he was not very impressed by Emerson's own explanation, which assumed that accessory pigments played a specific role in photosynthesis and directly initiated a second photochemical reaction.[178] As was mentioned earlier, this approach was in conflict with Duysens's own earlier finding that all the light energy absorbed had to pass through chlorophyll *a* if it were to be used in photosynthesis.[179] (The issue was eventually resolved when, in 1960, Govindjee & Rabinowitch (1960) showed that the two pigment systems activated by the longer and the shorter wavelengths both contained species of chlorophyll *a*, so that chlorophyll *b* did not need to be involved.)

In 1957, Duysens thought that the so-called "inactive" (non-fluorescent) part of chlorophyll *a*, which he had identified in his fluorescence studies (see p. 360), might be the key to solving the puzzle: under certain conditions, this "inactive" portion of chlorophyll *a* might possibly be triggered to participate in photosynthesis, through the effect of side reactions initiated by the fluorescing, "active" portion of chlorophyll *a*. Thus, Duysens set out to investigate in more detail the functions of the two different types of chlorophyll *a*, which he had already identified in 1952. Ironically, this was exactly the same research question that Rabinowitch had originally chosen for him, when Duysens spent a year in the Urbana laboratory in 1953. At the time, however, no promising experimental approach was available, so that Duysens turned to examining the kinetics of potential electron transport components instead.[180] In 1958, the situation had changed, and Duysens decided to examine the action spectra for cytochrome oxidation and TPN reduction in the presence and absence of short and long wavelength background illumination. The first results of these studies were reported in 1960 at the Third International Congress of Photobiology held in Copenhagen, Denmark, and caused considerable excitement in the audience: contrary to expectations, at 560 nm a *low* cytochrome oxidation yield was found (although the photosynthetic yield was high), while a *high* cytochrome oxidation yield was identified in the region of 680nm.[181] This switching of the oxidation state of a cytochrome when illuminated at different wavelengths was most surprising, and inexplicable, if one assumed that there was only one photoreaction in photosynthesis. Duysens proposed the following explanation:

> I postulated the existence of two major photosystems, 1 and 2. System 1 contained the weakly fluorescent chlorophyll *a*, formerly said to be inactive, and oxidized cytochrome; system 2 contained the fluorescent chlorophyll *a*.

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the findings of Govindjee et al. (1960*b*) and Govindjee, Owens & Hoch (1963) proved this to be . On Blinks's life and work, see, e.g., Thorhaug & Berlyn (2009).

[178] See Emerson & Chalmers (1958) and several talks given at the National Academy of Sciences.

[179] See Duysens (1989), p. 72.

[180] See Duysens (1989), p. 69.

[181] See Duysens (1961).

> An interaction between the two systems was shown by the different kinetics of cytochrome oxidation at different actinic wavelengths.[182]

Duysens recalled that in the discussion following his Copenhagen talk, Charles Whittingham asked him whether these findings "supported the scheme proposed by Hill and Bendall in *Nature*". Duysens said he did not know, since he had not seen the paper yet; but he rushed back to his laboratory to finish the final experiments and to establish "that cytochrome oxidized by 680nm background, mainly exciting system 1, was reduced by superimposing 560nm light, mainly exciting system 2". The photooxidation and reduction of the cytochrome occurred with a reasonable quantum yield, which, finally, "indicated that the 2 systems acted in series to produce complete photosynthesis".[183]

These impressive results were published in Duysens, Amesz & Kamp (1961). Therein, it was demonstrated that Duysens's system 2 could specifically and differentially be inhibited by DCMU.[184] The action spectrum of system 1 was found to be very similar to the spectrum of TPN reduction in cells, which suggested that the latter process was driven by system 1. At the same time, system 1 directly or indirectly oxidised the cytochrome (with predominant activity at 680 nm), while system 2 (with predominant activity at 560 nm) reduced the cytochrome. This now elegantly explained the Enhancement Effect found by Emerson, as Duysens, Amesz and Kamp underlined: "If the algae are illuminated with both wavelengths simultaneously, then the rate of photosynthesis will be higher than the sum of the rates at each wavelength separately, because by simultaneous illumination the excess absorptions supplement each other."[185] This was possible because of the overlapping absorption spectra of the two photosystems. The two functions of systems 1 and 2 were graphically summarised in a "hypothetical scheme", as the authors called it (see fig. VI.13.) Further evidence of the accuracy of this suggestion was drawn from its explanatory power with respect to related phenomena that had so far lacked any satisfactory interpretation. Studies from other laboratories had shown, for example, that, even when photosynthesis was inhibited by DCMU, the photoreduction of either carbon dioxide or TPN was still possible under certain conditions. "This indicates that these photoreductions proceed via system 1 solely," Duysens, Amesz and Kamp concluded, since DCMU only inhibited system 2.[186] On the other hand, the authors mentioned how their suggestion was in agreement with the important finding presented by Rajni Govindjee, Jan B. Thomas and Rabinowitch in 1960: "The observation that also the Hill reaction of *Chlorella* with quinone shows an 'Emerson Effect', indicates that both systems 1 and 2 participate in the Hill reaction."[187]

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[182] Duysens (1989), p. 72.

[183] All quotes: Duysens (1989), pp. 72–73.

[184] "DCMU" is (to this day) the abbreviated name for the herbicide 3-(3,4-di-chlorphenyl)-1,1-dimethylurea, which very effectively inhibits photosynthesis.

[185] Duysens et al. (1961), p. 511.

[186] Duysens et al. (1961), p. 511. Cited are the studies Bishop (1958) and Vernon & Zaugg (1960).

[187] Duysens et al. (1961), p. 511. The cited paper is R. Govindjee et al. (1960*b*). The Enhancement Effect was also found to be present in NADP reduction, as established in Govindjee, Govindjee & Hoch (1962) and Govindjee, Govindjee & Hoch (1964); a two-light effect in fluorescence excitation

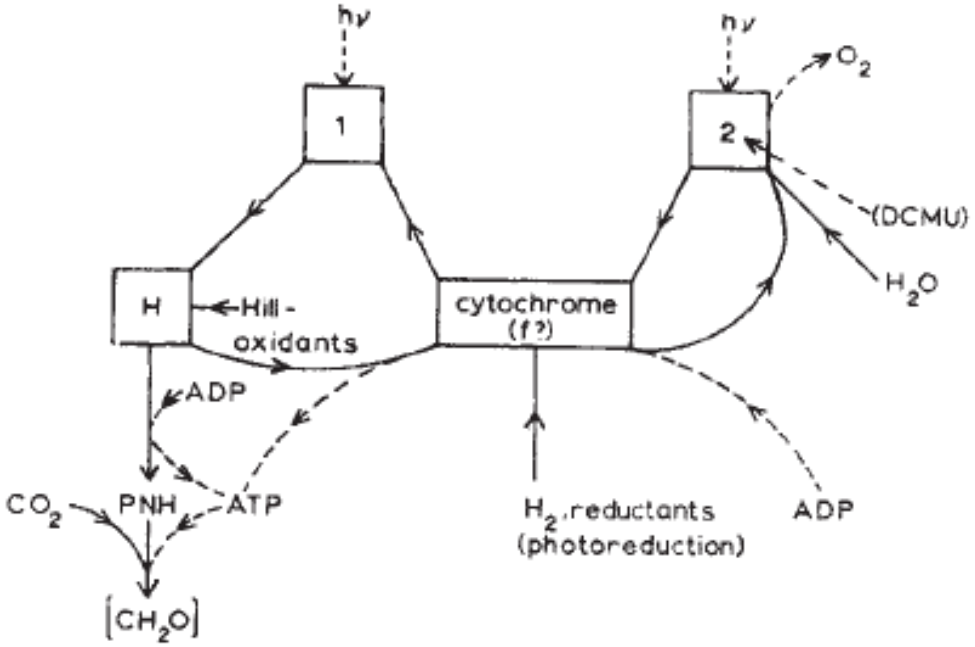

Fig. 3. Hypothetical scheme showing functions of systems 1 and 2 in photosynthesis

Figure VI.13: Duysens (1961), p. 511.

Like Hill and Bendall before them, Duysens, Amesz and Kamp considered their model a "working hypothesis", which was certainly incomplete and might require modification. Yet, they also emphasised the strengths of their proposal: "At present it explains most experiments known to us in a simple and plausible way." Finally, Duysens and his co-authors could not refrain from alluding to the paper by Hill and Bendall, albeit without actually citing it: "A more detailed report is in preparation, in which we will discuss partly similar but less detailed and experimentally less supported hypotheses concerning the role of the photosynthetic pigments which have been proposed by other authors."[188] Duysens, most probably, was understandably upset by the fact that a paper that had failed to acknowledge earlier work of his (and which was based on purely thermodynamical considerations), might deprive him of his credit for prior discoveries.

5.4 Two Light Reactions, One Pigment

It was described earlier how Bessel Kok had discovered a special pigment in higher plants, algae and cyanobacteria, which he had called P700, and how he had found, in the cyanobacterium *Anacystis nidulans*, an antagonistic effect of red versus orange light on this pigment: while the pigment was oxidised in red light, this oxidation was reversed when the cells were subsequently illuminated with orange

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was demonstrated in Govindjee, Ichimura, Cederstrand & Rabinowitch (1960*a*). See also Myers (1971) for a review of enhancement studies in photosynthesis up to then.

[188] Duysens et al. (1961), p. 511.

light. Already in 1959, Kok interpreted this finding as being related to the Emerson Enhancement Effect. He cautiously stated that a pigment which had an absorption band at 700nm was ideally suited to be the "final light sink in photosynthesis", and dared to speculate on a necessary alternation of different wavelengths effecting two different photochemical responses, in order to make photosynthesis work at its full efficiency (see quotation above, p. 366).[189] Kok continued to think along these lines, and later in 1959, at the Ninth International Botanical Congress held in Montreal, Canada, he presented a paper entitled "Does photosynthesis require the interaction of two photochemical steps?" And his answer was decidedly positive. In the paper's abstract Kok announced the discussion of a hypothesis "which conceives a cycle of two photochemical acts in photosynthesis".[190]

Kok had found that the oxidation of P700 was brought about by the light absorbed by chlorophyll *a*, whereas in the subsequent restoration and reduction processes it was mainly light absorbed by phycocyanin that was active. Having presented the relevant data, Kok put forward the conclusion that "two antagonistic light reactions and at least one dark step determine the concentration of (the absorbing form of) P700".[191] A major fraction of the light energy absorbed by the chlorophyll seemed, in fact, to pass through P700, which could account for the final drop in the light energy resonance transfer because of its absorption spectrum and concentration level. "If indeed P700 does play such a key role", Kok concluded, "the finding of two opposite effects of light, sensitized by different pigment entities, leads to the hypothesis of a cyclic process, driven one way by quanta received by chlorophyll *a* and back by quanta received by accessory pigment[s]."[192] According to Kok, the Emerson Enhancement Effect as well as the finding of chromatic transients supported this conclusion (since the two photo-reaction scheme could quite easily explain them). Together with the biochemist George Hoch, Kok elaborated these studies and reported the outcome at the "Light and Life" symposium, held in March 1960 at the Johns Hopkins University in Baltimore (US).[193] The authors again asked, whether photosynthesis was actually driven by two light reactions, and their answer was, again:

> The observations discussed in the above sections strongly indicate the occurrence of two different light reactions: the first sensitized by chlorophyll *a* and a direct bleaching of "P700"; the second sensitized by accessory pigments acting indirectly via the mediation of dark steps and restoring "P700".[194]

[189] See Kok (1959).

[190] See Kok (1961*a*) for the publication of an extended abstract. It is thanks to the careful research of Govindjee that this paper of Kok's, which had long been overlooked, finally became widely recognised as a very early concept of a photosynthesis model that includes two photochemical reactions; see Govindjee (2006).

[191] Kok (1961*a*), p. 1072.

[192] Quoted from Govindjee (2006), p. 153.

[193] See McElroy & Glass (1961) for the proceedings of the symposium, in which not only presentations but also subsequent discussions are documented. Jack Myers thought, in retrospect, that it was at this conference that the idea of two photochemical reactions being involved in photosynthesis started to dawn on photosynthesis researchers, with the contribution by Kok and Hoch being considered pivotal in this respect. See Myers (2002), p. 25.

[194] Kok & Hoch (1961), p. 407.

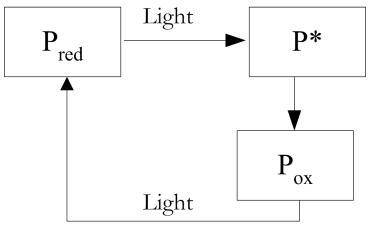

Figure VI.14: A proposal for the cyclic mechanism in which P700 is oxidised and reduced, and which involves two light reactions and one dark reaction. After Kok & Hoch 1961, p. 408.

Kok and Hoch clearly stated that "two light steps and at least one dark step must be involved in the behaviour of 'P700'."[195] A possible scheme for the cyclic process envisaged by Kok and Hoch is given in figure VI.14. From their data, Kok and Hoch also concluded that (in contrast to Kok's paper of 1959) phycocyanin was not essential for the process to function – chlorophyll *a* was able to sensitise photophosphorylation entirely by itself.

These were enormous advances on the way to a better understanding of the photochemical events in photosynthesis. However, in his biographical memoir of Kok, Jack Myers noted that the audience at the 1960 conference was far from enthusiastic: "Bessel and George [...] had discovered and understood an important truth that no one else had yet seen. But the reception of their paper was a disappointment. From the record of discussion one would judge that they had dropped an egg instead of a bomb." Myers thought that Kok and Hoch had shown too many experimental details, so that the main argument was almost lost.[196] This is important to keep in mind: even though, with hindsight, it seems that, by 1960, enough compelling evidence for the two light reaction hypothesis had been accumulated from a number of different studies, the hypothesis was far from being universally accepted or even self-evident. Everyone had grown up with the idea that there was only one type of primary photochemical process, and it was not easy to dispel this deeply rooted belief.

5.5 Montreal, 1959

In his re-evaluation of the contributions of the 1959 botanical conference held in Montreal, Govindjee drew attention to the fact that Myers also presented a paper in which he linked Blinks's chromatic transients to the Emerson Enhancement Effect, demonstrated the equivalence of their action spectra and, thereby, likewise postulated the existence of two light reactions, one of which he thought (erroneously) was specifically associated with chlorophyll *b*.[197] And, finally, Rabinowitch also

[195] Kok & Hoch (1961), p. 409.

[196] Myers (1987), p. 134.

[197] See Myers (1961) for the abstract of the pertinent paper; see Govindjee (2006), p. 154, for the discussion of Myers's contribution. See also the related and more extended papers Myers & French (1960*a*) (accepted 23 July 1959; published in March 1960) and Myers & French (1960*b*) in which the authors showed that the Enhancement Effect took place even with a dark interval of 0.6 seconds between the two different light beams. That the imposition of a certain time lag

discussed again the possibility of two photochemical events at this conference: after Emerson's tragically early death in February, Rabinowitch went to the Montreal conference in Emerson's place and presented data from Emerson's estate, together with Rabinowitch's own interpretation.[198] Rabinowitch noted that the "simplest" explanation for the Enhancement Effect was "to postulate that two (or more) different primary photochemical processes are involved in photosynthesis, and are preferentially sensitized by the several pigments". Rabinowitch maintained "that each hydrogen atom (or electron) must be photoactivated twice on its path from the ultimate donor, water, to the ultimate acceptor, carbon dioxide, and that each of these two steps is preferentially sensitized by one or the other pigments".[199] Rabinowitch proposed that

> "chlorophyll *a*, carrying the transferred quanta [received from auxiliary pigments], must be postulated to bringing about preferentially a primary photochemical process different from the one caused (preferentially) by the long-wave quanta absorbed by chlorophyll *a* itself. To make this plausible, one could postulate, for example, the existence of two types of chlorophyll *a* complexes in the cell, e.g., such associated with a reductant and such associated with an oxidant, and assume that one of these two types is closer to the auxiliary pigment than the other.[200]

This idea was soon backed up by sound experimental evidence: the different functions of two different types of chlorophyll *a* were established in Govindjee & Rabinowitch (1960), and similar findings were presented in French (1961). In their contribution to the "Light and Life" conference, Rabinowitch and Govindjee speculated that the primary photochemical process in photosynthesis might consist of two steps: whereas one type of chlorophyll *a* could enable both steps, the other type was restricted to only one of these steps.[201]

5.6 Fluorescence Studies

The third approach that led to the conclusion that two different photochemical reactions were involved in photosynthesis was the study of fluorescence. It was mentioned in Chapter III (p. 110) that the German chemist Hans Kautsky was the first to interpret systematically the course of chlorophyll fluorescence as an indicator of the photosynthetic mechanism. While the research focus of Kautsky and his group shifted to other topics, such as the singlet state of oxygen and the chemistry of silicon oxides, chlorophyll fluorescence remained on Kautsky's agenda and he continued to publish on the subject during the 1930s. He made a noteworthy

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between the light beams favoured the Enhancement Effect was already recognised qualitatively by **?**).

[198] See Emerson & Rabinowitch (1960) for the resulting publication; Govindjee (2006), p. 157, cites the abstract written for the Montreal conference.

[199] See Emerson & Rabinowitch (1960), p. 482.

[200] Emerson & Rabinowitch (1960), p. 484.

[201] Rabinowitch & Govindjee (1961), p. 385: "The enhancement of the quantum yield in the far red by auxiliary light suggests that the 'inactive' form Chl *a* 690 is not entirely inactive, but requires a balanced co-excitation of an 'active' form to contribute fully to photosynthesis. It seems natural to think in this connection of the possibility that the primary photochemical process in photosynthesis might consist of two steps. Excited Chl *a* 690 may be able to bring about only one of these steps, while excited Chl *a* 670 may be able to sensitize both of them."

contribution in 1943, when, together with Ulrich Franck, he revisited the theme on the basis of improved technique, using visible light and the automatic registration of fluorescence.[202] In their study, they interpreted for the first time the course of chlorophyll *a* fluorescence as indicating the existence of altogether four primary photochemical reactions (corresponding to a minimum quantum requirement of four, as was still standard at the time). The first "drop" in fluorescence (*erste Depression*) was explained by the "unique mechanism in the plant" that involved three coupled light reactions, which combined with subsequent dark reactions to form reaction cycles.[203] A fourth light reaction was then envisaged to explain the subsequent rise in fluorescence. This notion was criticised by other authors; it was pointed out, for example, by Wassink (1951) that the drop might also be caused by side reactions.[204]

In 1960, Kautsky revisited this theory, together with Walter Appel and H. Amann, the result of which was the paper Kautsky, Appel & Amann (1960). Based on new experimental evidence, the authors revived the attempt to recognise two photochemical reactions as the underlying causes for the course of chlorophyll *a* fluorescence. Their study was based on experiments undertaken with a much improved technique (developed since 1955), which allowed them to detect fluorescence events with a time resolution of 10 milliseconds. By this means, they were able to confirm that photosynthesis acted through "sensitising areas" (*Sensibilatorbereiche*) of a few hundred chlorophyll molecules or more, which transferred light quanta to a specific acceptor molecule (A_0), while, at the same time, quenching fluorescence.

Hence, there seemed to be a photochemical reaction $A_0 \rightarrow A_1$, while the back reaction was not light dependent but rather a dark step that involved another compound, B_1. Now, the interesting part of the argument was that, according to Kautsky et al., the shapes of the fluorescence curves were incompatible with the assumption that this compound B_1 was already present before the onset of illumination. The curve "rather indicated that B_1 was formed upon illumination".[205] The authors thus concluded that there was a second photochemical reaction $B_0 \rightarrow B_1$. Further kinetic considerations seemed to support this assumption. Note, however, that it is not entirely clear how widely and internationally this paper by Kautsky, Appel and Amann was received and whether it was recognised at all as an interesting and relevant contribution – the contemporary papers discussed in this book do not refer to it; likewise, the paper by Kautsky et al. also contained no references to other publications that appear relevant in this context. Indeed, Kautsky seems to have been unaware of the heated debate that was taking place as to whether or not there were two different pigment systems in photosynthesis. It is possible that in this case language acted as a barrier as the paper was written in German and was published in a German journal; and perhaps the actors themselves did not realise some of the similarities that, with hindsight, are so obvious.

More influential, therefore, was the study of the two-light effect on fluorescence yields that was independently and simultaneously undertaken at Urbana. Govin-

[202] See Kautsky & Franck (1943).

[203] Kautsky & Franck (1943). p. 195.

[204] See also Govindjee (2004*a*), p. 16, and Govindjee (1995), p. 138.

[205] Kautsky et al. (1960), p. 282. German original: '[Diese Kurvenform] weit vielmehr auf ein Entstehen von B_1 während der Belichtung hin.

djee et al. (1960*a*) found that light of different wavelengths had different effects on the fluorescence yield: the total chlorophyll *a* fluorescence caused by being illuminated with far-red *and* red light was smaller than the sum of the fluorescence intensities when the cells were illuminated with individual beams. Fluorescence excited by red light (absorbed by the short-wavelength pigment system) hence seemed to be quenched by illumination with light of far-red light (absorbed by the long-wavelength pigment system). This was regarded as additional evidence of the existence of the two light reaction, two pigment system of photosynthesis.[206]

5.7 New Flashing Light Experiments

Another group that simultaneously investigated the peculiar nature of the primary processes in photosynthesis was the research team of the German biophysicist Horst T. Witt. A student of the renowned theoretical physicist Robert Pohl at the University of Göttingen in Germany, Witt received his PhD in solid-state physics in 1950.[207] At Göttingen Witt began to develop an interest in the physical foundation of biological phenomena – a field that would soon be called "molecular biology" or, alternatively, with a slightly different focus, "biophysics".

Witt started working seriously on oxygenic photosynthesis when the German physical chemist Karl Friedrich Bonhoeffer offered him a laboratory at the Max Planck Institute of Physical Chemistry in Göttingen in 1952. Two methodical problems needed to be addressed if one wanted to find out more about the primary photochemical processes: first, one had to develop techniques that were able to precisely measure the absorption changes (as the largest share of the pigments were chemically inactive, the absorption changes brought about by the redox reactions were extremely small, so that the usual instruments failed to give accurate quantitative results); and second, these measuring techniques had to beable to deal with the extremely high speed of the reactions (almost all the pertinent reactions were faster than 10 milliseconds – in fact, many were suspected to be in the range of micro- and nanoseconds). Witt succeeded in detecting these changes with his flashing light spectroscopic methods, which greatly increased the sensitivity and the time resolution of photosynthesis studies. In 1955, at the second Gatlinburg conference , he presented this technique and at the same time introduced himself to the most influential photosynthesis researchers of the period.[208] With the help of this method, in 1961 Witt and his collaborators found the following:

- Upon excitation with light$_1$ (710 nm) cytochrome *f* was oxidised and stayed in this state for seconds.
- After excitation with light$_2$ (670 nm) an unidentified component X was oxidised to XO.

[206] See also Govindjee (1995) and Govindjee (2004*a*). The same phenomenon was demonstrated by Butler (1962), who obtained even more convincing data. Duysens & Sweers (1963) provided the current interpretation, including the postulation of an intermediate that had been preliminarily termed "Q".

[207] For biographical information on Witt, see, e.g., Junge & Rutherford (2007), Jaenicke (2007) and Renger (2008). Witt (1991) is an autobiographical account of his life and work; Junge (2005) describes the later discoveries (after 1966) achieved in Witt's laboratory from the perspective of one of Witt's closest collaborators

[208] A survey of Witt's methods and findings up to 1959 is provided by citeasnounWitt1960.

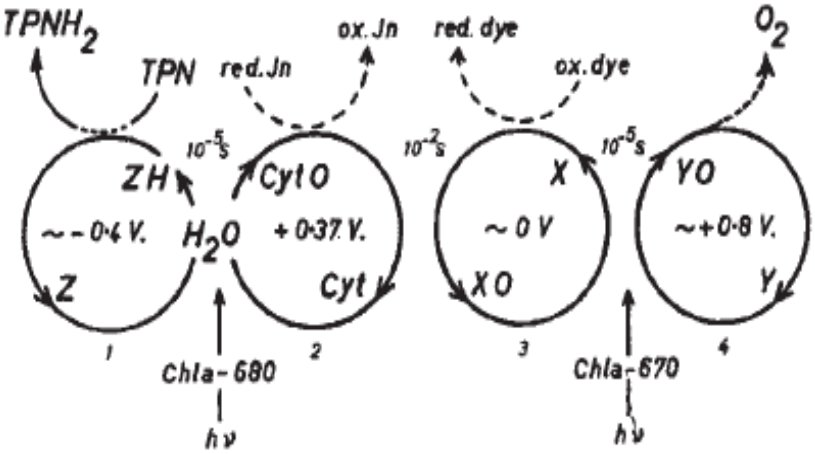

Fig. 2. Reaction pattern of photosynthesis

Figure VI.15: A proposal for the cyclic mechanism of photosynthesis in Witt et al. 1961, p. 194.

From these findings, Witt et al. concluded (at almost the same time as Kok and Hoch as well as Duysens, Amesz and Kamp) "that photosynthesis is triggered by two different photochemical reactions: oxidation of cytochrome by Chl*a*-680 and reduction of XO by Chl*a*-670".[209] Based on the absorption changes and other pieces of evidence, Witt et al. additionally suggested that the reaction XO $\rightarrow$ X might be the reaction of plastoquinone to hydroquinone.[210] Unfortunately, Witt's group was unable to find evidence for the reduction of cytochrome, which one would have expected to be coupled to the oxidation of X. Nevertheless, in the same year (1961), it was established, through independent studies undertaken by Kok on the one hand and Witt et al. on the other, that P700 was a chlorophyll *a* molecule and the primary electron donor in the long-wavelength photosystem.[211]

5.8 The Z-Scheme

Over the course of the years the two photochemical reactions, two photosystems model has come to be called the "Z–scheme" of photosynthesis. Today, it features

[209] Witt, Müller & Rumberg (1961*a*), p. 194.

[210] The original publications were Witt et al. (1961*a*) and Witt, Müller & Rumberg (1961*b*). Cf. also the retrospective analysis of the episode in Witt (1991), pp. 61–63. Plastoquinone is omnipresent in higher plants, and by the end of the 1950s researchers had suspected that it was somehow involved in the electron transport chain; this was later confirmed by studies undertaken in Witt's laboratory. See, e.g., Bishop (1959) for the first clear statement about plastoquinone's possible role; Klingenberg, Müller, Schmidt-Mende & Witt (1962) was able to corroborate the theory.

[211] Kok (1961*b*) and Witt et al. (1961*b*).

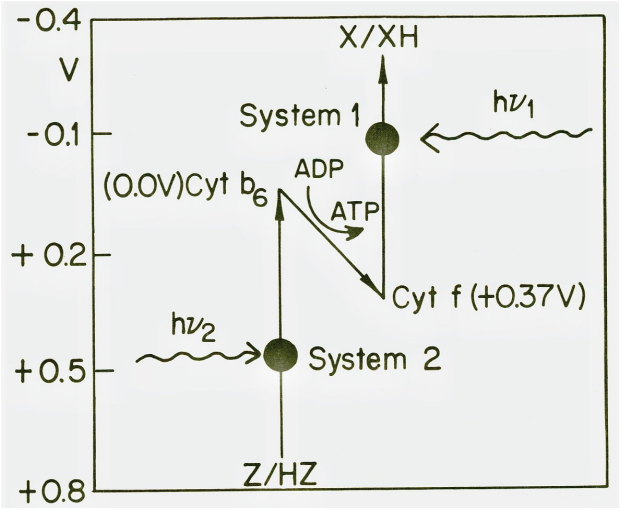

Figure VI.16: The "Z-scheme" of photosynthesis, reproduced from Rabinowitch (1963), p. 114.

prominently in secondary school biology textbooks, although current standard representations often do not make it clear why it was called the "Z–scheme" – neither is this apparent from the diagrams used by Hill and Bendall. In its early years of existence, rather different means of representation were used to represent the two photosystem model. A version that was quite close to the arrow scheme by Hill and Bendall, albeit with a different orientation of the vertical axis, was used by Rabinowitch in 1963, in his contribution to the conference on "Photosynthetic Mechanisms of Green Plants", held at Airlie House, Virginia (see fig. VI.16). Already then, Rabinowitch mentioned that similar schemes, "presented vertically, horizontally, in zig-zags, on circles or curlicues", had been presented by several authors in recent years.[212] Rabinowitch himself settled on a vertically oriented scheme, displaying only a minimum of information. (Note that a vertical orientation leads to a more intuitive representation of the spontaneous, thermodynamically "downhill" reaction between cytochromes b_6 and f: the reaction then also goes "downhill" in the picture.) Compare this, however, with the later versions, which are reproduced in figures VI.17 and VI.18: if the axis with the redox potentials is horizontally oriented, a neat letter"Z" appears in the scheme.[213] David Krogmann (2000) remembered that in the 1960s "there was heated discussion of whether the arrows depicting photoacts should point up or down and whether to rotate the Z clockwise or counterclockwise in what came to be known as the Z scheme".[214]

[212]Rabinowitch (1963), p. 115.

[213]The figures were taken from Govindjee & Govindjee (1975), p. 27, and Demeter & Govindjee (1989), p. 123.

[214]Krogmann (2000), p. 115. Krogmann himself mentioned "a Gordon Conference in the early 1960s, in Tilton, New Hampshire"; however, the first Gordon Research Conference on Photosynthesis was held in 1969, so he may have confused the Gordon Conference with the 1963 conference held at Airlie House, Virginia.

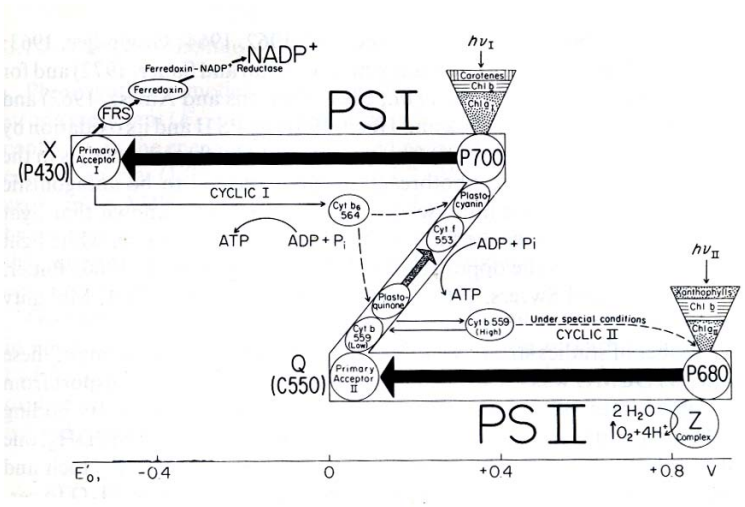

Figure VI.17: The "Z-scheme" of photosynthesis, in a version by Govindjee & R. Govindjee 1975, p. 27.

This model and its current standard form of representation became a matrix that synthesised most of the available evidence on how the light reactions in photosynthesis work, and which could be expanded and completed as time went by. In this model, photosystem I (PS I) causes, on the one hand, the reduction of TPN (in the figure already named $NADP^+$) and eventually of carbon dioxide, as well as the oxidation of cytochrome and P700; whereas photosystem II (PS II) causes the reduction of the cytochrome and P700 as well as the oxidation of water, whereby oxygen is released. Both systems need to be in a sufficient and balanced excited state for photosynthesis to occur efficiently, and this excitation straightforwardly explains the Emerson Enhancement Effect. In the years that followed, many more components of the electron transport chain were identified, and other pieces of evidence were accumulated, all of which were explained by moderately expanding the model (by inserting new intermediates, for example), or, at the very least, they were compatible with it. This made it ever more probable that the model was, in principle, on the right track and it was soon almost universally accepted – although, of course, its intricate details remain controversial to this day.

6 Reflections on Convergent Research Pathways

It is clear from what has been discussed in this chapter that the establishment of the Z-scheme model of the photosynthetic light reactions, which included two sequential photochemical reactions initiated by two different pigment systems, was arrived at almost simultaneously by a number of research teams working in Europe and the US, starting from very different angles, and using different methods and approaches. This contrasts starkly with the discovery of the cyclic pathway of the dark reactions in photosynthesis, which was so strongly dominated by the Calvin–Benson team at Berkeley (see Chapter V). No other research group had the manpower or infrastructure to even match the Berkeley group – rather, whoever

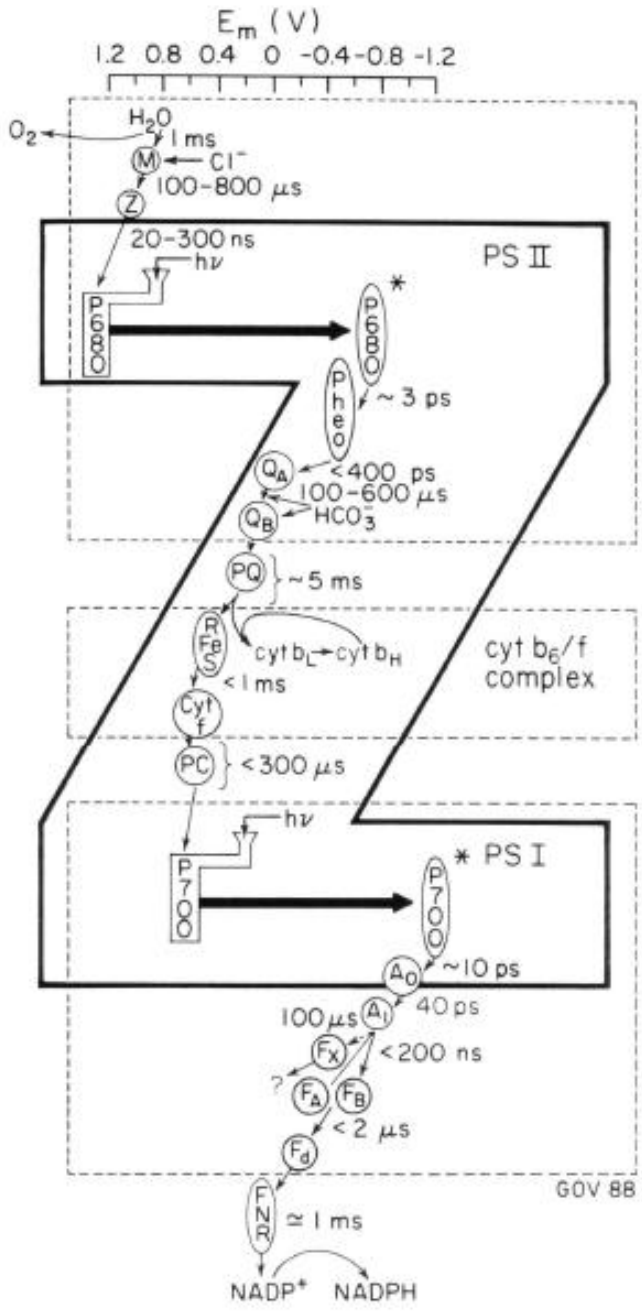

Figure VI.18: The "Z-scheme" of photosynthesis, reproduced from Demeter & Govindjee (1989*a*), p. 123.

felt that he or she might be able to contribute something, tried to collaborate with the Berkeley team. Thus, the divergent competencies required to reach the goal of finding the path of carbon in photosynthesis – not least through exploring even apparently far-fetched possibilities – was assembled in one place. A much more decentralised approach was adopted when it came to explaining the photochemical events of the process.

Most of the work examined in this chapter was carried out in the 1950s: progress started to accelerate around 1954 and culminated in the years 1959–60. Seen in retrospect, everything took off in 1951 when the Hill reaction was suddenly seen to be related to the reduction of carbon dioxide, through the finding (simultaneously in no less than three different laboratories) that chloroplasts were able to reduce TPN and, hence, provide one of the essential components for the reduction of carbon dioxide in the dark cycle. Although Emerson and others were sceptical about the significance of this finding, Hill found it immensely satisfying: finally, there was evidence that the Hill reaction was, in fact, not extraneous to photosynthesis but reflected an inherent part of the process. Only one year later, in 1952, was the plant-specific cytochrome f found, followed by cytochrome b_6. So that, by the time Duysens had established, around 1956, that cytochrome f reversibly changed its redox state upon illumination, Emerson had found the Enhancement Effect of illumination with two different wavelengths and Kok had found the antagonistic effect of red and orange light on P700, the eventual solution to the problem was only one small step away.

Although all the actors mentioned in this chapter shared the same research goal – to elucidate the primary photochemical processes of photosynthesis – it is obvious that they used very different means to reach this goal. The paper by Hill and Bendall, for example, was based on purely theoretical, thermodynamical considerations. The fact that Hill intended his paper to solve, in the first place, the cytochrome question might explain why he did not refer to Emerson's papers on the Enhancement Effect, of 1957 and 1958; for the argument developed in the Hill–Bendall paper, Emerson's finding was not essential. Yet, of course, the explanation of this effect was a strong argument for the accuracy and justification of the Hill–Bendall model; and it is hard to argue that Hill had not been influenced in his thinking by the discussion of the Enhancement Effect and its potential explanation. The same applies to Duysens's observation concerning the behaviour of the redox state of cytochrome f in the light, which likewise was not cited by Hill and Bendall. The only other person to suggest a two photoreaction model for photosynthesis without referring either to the Emerson Enhancement Effect or to other contemporary contributions to the debate on the primary processes in photosynthesis was Kautsky. From Kautsky's paper it is unclear how familiar he was with the photosynthesis research work being undertaken in other countries; it certainly seems that he was not very au courant with developments in England and the US.

With the exception of Hill and Kautsky, the Emerson Enhancement Effect had a decisive influence on most of the other actors' paths to the two photosystem model. In his paper of 1959, Kok admitted that he would not have considered the antagonistic light effect on P700 as fascinating as he did had he not suspected that

there was a rather direct connection to the Enhancement Effect – and it was in the Emerson Memorial Issue of the journal *Plant Physiology* that Kok cautiously noted, for the first time, the explanatory dimension of his observation.[215] It is not entirely clear from where Witt acquired his motivation; but Duysens, it is clear, was strongly driven by the incentive to explain the Emerson Enhancement Effect (as well as by Blinks's finding of chromatic transients). On second thoughts, it is not surprising that Emerson's finding of 1957 was so influential. The effect was completely unexpected, so that it aroused considerable interest in itself; furthermore, in order to explain the effect Emerson himself had suggested, as early as 1957, that two different photochemical reactions might be involved and that they were initiated by different pigments. Thus, the main elements of the solution had not only been "in the air", that is, somehow to be intuitively inferred from current discussions, but had been explicitly formulated at a very early stage. Already in 1959, Rabinowitch and others of the Urbana group elaborated and improved upon Emerson's thoughts; and envisaged two different processes involving two different species of chlorophyll *a* that absorbed at different wavelengths (which were demonstrated to exist, mainly through the work done by Govindjee). Why the photosynthesis collective did not react immediately and celebrate the birth of a Z-scheme-like model one year earlier is not entirely clear; even in March 1960, a very similar suggestion by Kok and Hoch was still received with much reservation. The small group of experts directly involved in the study of the issue, had definitely been won over by then, but a lot more work needed to be done before the Z-scheme was generally accepted. However, by 1963, the general accuracy of a Z-scheme-like model was no longer seriously disputed. There was disagreement about the details; yet most of the alternative suggestions, repeatedly published, for example, by Arnon's laboratory at Berkeley, did not survive for long. The main argument in favour of the Z-scheme was its explanatory power. The Z-scheme was able to explain, with recourse to only one general assumption (namely, the existence of two photochemical reactions implemented in two different pigment systems) phenomena ranging from the Enhancement Effect to the light-induced redox changes of cytochrome *f* and the chromatic transients of oxygen exchange. And this was hugely persuasive.

Duysens himself believed, in retrospect, that once the appropriate methods were known (such as absorption difference spectroscopy) and some important parts of the jigsaw pieced together (such as the discovery of light-induced redox changes in cytochromes and P700), arriving at a Z-scheme-like model was only a matter of time and could not be credited to any specific person.[216] This is entirely convincing when looked at from the collective's perspective: if all the acquired knowledge is pooled, it is to be expected that, at some point, someone will successfully apply the available techniques and concepts to photosynthesis research. It is equally true, however, that each individual who, around 1960, arrived at the conclusion that there are two photochemical reactions was exceptionally well prepared to do so, each in his own distinctive manner. Although it is extremely important to have access to appropriate methods, and the introduction of new methods to the

[215] See Kok (1959).
[216] See Duysens (1989), pp. 77–78.

field of photosynthesis studies repeatedly proved immensely fruitful and valuable, methods and techniques are not everything. Researchers have to know how to use them and how to conceive of the kinds of problems that can be solved using them. They need to realise the new techniques potential and how specific methods can help one to reach a personal goal. If nobody knows how one might benefit from the new methods, even those methods that might enormously propel some field of knowledge will prove to be worthless. Measuring photochemical efficiencies, for example, had long been a standard method in photochemistry; but it was Warburg and Negelein who introduced these approaches to photosynthesis studies; and they only did so because they seemed to be an appropriate way of reaching their goal.

Finally, let me draw attention to the fact that Kok, Hill and Duysens all believed that it was necessary to present their own suggestions as tentative "working hypotheses" that needed further clarification and modification. One would have thought that such a disclaimer was superfluous, since the same is true of every model hypothesis, but it reveals the enormous degree of uncertainty that still prevailed around 1960. Nobody was certain that they had hit upon the right solution, however convincing it might have appeared at first glance. Indeed, it is a rather surprising aspect of the history of photosynthesis that time and again those solutions that at first appeared less probable, turned out, in the end, to be accurate, whereas those alternatives that at first glance looked almost obvious were eventually dropped. For example, for a long time it was taken for granted that carbon dioxide was the source of photosynthetic oxygen – given the stoichiometry of the summary equation, this was more than probable – although, in the end, it transpired that water was the source of photosynthetic oxygen. And far into the 1930s, it was assumed that the light reaction in photosynthesis was directly related to carbon dioxide reduction, although this also turned out to be erroneous. As late as October 1959, at a conference on bioenergetics, William Arnold was infuriated that participating physicists rejected potential mechanisms of the photochemical events in photosynthesis as they found that the mechanism under debate did not seem very *probable*. "Any statement about 'probable' might be alright in Havana, where you ould make money on it; but, this is a meeting on bioenergetics, and when it comes to biology, this is simply an extraneous consideration of no importance," Arnold sharply commented.[217]

Taking stock of the entire course of events, I could not agree more. Photosynthesis is in itself a most improbable process. No chemist or physicist would ever have come up with a system that generates its energy from oxidating water and stores it by way of carbon dioxide reduction – all this at room temperature to boot. Yet, it is a process that operates extremely well: it is, after all, the basis for all life on earth. However, in order to explain such an improbable process, scientists had to explore even the most improbable of modelling approaches; and right up until the final solution was reached, it was never completely foreseeable which of the available options would, in the end, prevail.

[217] Arnold (1960), p. 324.

Chapter

EPILOGUE

The discovery process initiated by the nineteenth-century chemists to explain photosynthesis thus came to a preliminary conclusion around 1960, largely thanks to the engagement of far-sighted biophysicists. The starting point of the process was the attempt to explain photosynthesis by pointing to a handful of causally relevant factors; the point of termination was a sophisticated, molecular-level mechanism. One could describe this as a journey out of darkness into the light, albeit with many shady periods in between: intermittent sunny intervals but mostly fog and clouds. Simply put, it is the story of a complex problem and the long and winding path to its solution. The problem – how to explain the light-driven production of carbohydrates and oxygen from carbon dioxide and water in green plants – had troubled scientists for more than a century. The solution included an intricate cyclic path, the prerequisites of which – reducing equivalents and chemically usable energy in the form of adenosine triphosphate (ATP) – were produced during the course of two photochemical reactions operating in series and dependent on two different pigment systems. (Of course, one has to add that, from the perspective of modern-day science, work on the "real" problems of photosynthesis only started in 1960.)

The story of how this solution was reached was described in this book, with an emphasis placed on the internal dynamics of the modelling process. The modelling of a complex phenomenon, such as photosynthetic carbon dioxide assimilation, was presented as being the work of a collective enterprise, in the sense that research groups pursuing similar research goals *cooperated informally* with one another. No central agency organised this cooperative venture; however, a neat division of labour arose, in so far as different sub-collectives selected their own sub-goals, which appeared interesting, relevant and, in all probability, attainable within a realistic time span with the means at hands. Most scientists specialised in certain experimental techniques and applied them to a limited range of issues within photosynthesis research, at the same time keeping a close eye on the (complementary) work of other researchers in the field. The more complex the issue turned out to be, the more sub-goals were identified and the more diversified the collective became. By the 1940s, a clear-cut distinction had developed between those researchers trying to elucidate the so-called dark reactions of photosynthesis, using radiotracer techniques and chromatography, and those studying the light reactions of photosynthesis, using manometry and spectroscopy. And, although research groups within these two divisions might pursue identical research goals (such as attempting to determine the maximum efficiency of photosynthesis), very few of these groups used the same method on the same organism, and so were not directly competing with one another. To be sure, there were attempts to reproduce certain experimental results. Yet, most of the time, if the validity of the data had been demonstrated to be sufficient, then the data were used by other scientists to make headway in solving the main problem.

This was done by establishing the causally relevant factors of the complex model that the scientists were trying to construct; in this sense, the modelling process was also presented in this study as the *search for an accurate and adequate causal graph*. It had to be accurate in so far as ideally all the causally relevant factors were based on the conclusive outcome of difference tests; and it had to be adequate in so far as the resolution of factors had to correspond to the capacities of contemporary methods and to the conventions of the field. One should, of course, add that the graph (or the model) also needed to adhere to accepted theory and to be able to explain consistently as much of the relevant data as possible. Constructing these causal graphs was far from easy. The difference tests performed in experiments yielded knowledge on causally relevant factors, but it was an altogether different matter to integrate these results into a larger context. Although the diagnosis of a causal relevance is essential – indeed, it is the principal objective of experimental research – a causally relevant factor does not automatically lend itself to being clearly translated into a causal graph. For example, although it had become obvious relatively early in the twentieth century that photosynthesis involved at least one thermochemical reaction in addition to the photochemical reactions, for many years scientists could not agree on which partial processes of photosynthesis were -driven by light and which were not.

Several strategies were identified in order to bridge the gap between establishing the causally relevant factors and assembling the intended causal graph. One of the most important heuristic techniques identified in this study was the *transfer of causal knowledge* from one discipline to another or, in other words, the tentative classification of a new situation as being a model of a type of situation that has already been elucidated. This was particularly useful in cases where it was impossible to undertake difference tests to find out more details about the involved causally relevant factors, mainly because of the lack of appropriate methods. As the internal functioning of metabolism was still a complete mystery in the nineteenth century and nothing was known about photosynthesis except for the identity of the raw materials and the end products, scientists had no other option but to reason along these lines: identify as many reaction paths as possible of the raw materials and try to establish which of them occur in photosynthesis. Succeeding generations of photosynthesis researchers later criticised these decades, in the second half of the nineteenth century, as a period of spurious speculation, although in doing so they overlooked the fact that much valuable knowledge was accumulated about the different types of reactions and partial processes during these years. Indeed, the transfer of causal knowledge continued to be an extremely powerful tool, for example in elucidating the course of the dark reactions of photosynthesis: the compounds were identified by means of the new technique of paper chromatography, but how they interacted had to be inferred from the body of knowledge assembled about these compounds in other systems.

This type of reasoning is sometimes referred to as “reasoning by analogy” or “analogical reasoning”. Yet, although these terms might appear suggestive, they do not, however, explain why it can sometimes be highly successful (and, at other times, highly misleading). Translating the theme of analogy into the framework of causal reasoning is a much more helpful way of clarifying the matter. Reasoning

along these lines is appropriately conceived of as applying the causal knowledge that has been obtained in one situation to other situations that have been judged to be of the same type. One could describe this strategy as a type of "inference to the best explanation". If phenomena are observed whose causes are known in similar contexts, one might suppose that the action of these causes under the conditions investigated would satisfactorily explain the problem at hand. (One can easily recognise Newton's second Rule of Reasoning here.) Methodologically, this is justified by the fact that causal knowledge always concerns types of events, not only individual tokens. Thus, if it seems that (almost) all the same factors under like circumstances produce (almost) the same effects, one may assume, as a rule of thumb, that these processes belong to the same type and, hence, are governed by the same causal framework.

The transfer of causal knowledge is an essential element of scientific modelling. It can concern single factors or full modules, that is, branches of the causal graph. Of course, mistakes can be made when knowledge is transferred, so double-checking is necessary. For this very reason, a theoretical suggestion based on the transfer of causal knowledge was frequently accompanied or closely followed up by a phase of empirical investigation, in order to ascertain whether the assumed consequences did, in fact, hold. And it is interesting to note that, even if this empirical search did not go anywhere, scientists sometimes retained the tentative causal assumptions, particularly when the evidence from other quarters was strong and its explanatory power persuasive enough. The long-held belief in the accuracy of the formaldehyde model of photosynthesis (which was based on the knowledge gained in artificial systems), despite the failure of scientists to demonstrate that formaldehyde was formed in plants, is a case in point. Frequently, it was not the actors themselves, who, finding themselves in an impasse, actively sought to transfer knowledge from other fields to photosynthesis studies (although the intensive search of the literature for potential chemical intermediates of photosynthesis that Martin Kamen and Sam Ruben undertook when they started their tracer studies could be considered an exception to this rule). Rather, new pieces of knowledge were generally imported by scientists who were experts in other areas (such as nuclear physics, physiology and radiation chemistry) and had diverged from their original discipline to make a quick contribution to photosynthesis studies. Some of them returned to their main speciality, while others stayed in the field. The application of new methods and instruments was also frequently introduced to photosynthesis research by these *research opportunists*, as they were called in this study.

Another modelling strategy was found to be the way the causal graph was divided into modules – distinct branches, only some of which were investigated at any one time, that could be treated as a unit. This heuristic rule proved particularly useful once the causal graph under construction had reached a certain degree of complexity. *Modularising the graph* could serve different purposes: it was used to exclude certain sub-processes from the focus (such as the interaction of chlorophyll with light); to import full modules from other branches of science (see above); or to justify a scientist's own focus of research. Early on in photosynthesis research, for example, the synthesis of carbohydrates from formaldehyde was treated as a "module": it was accepted as a self-sufficient element, which most researchers

"grafted" onto their models without, however, rechecking the involved causally relevant factors. Naturally, one had to ensure that the newly grafted module was consistent with the rest of the model and that there was an appropriate point into which the module could be incorporated. But apart from these basic requirements, modularising allowed researchers to treat a phenomenon comprehensively without having to deal with every single element concurrently. The most extreme example of "modularisation", which eventually resulted in the construction of two distinct partial models, involved the separation of the dark reactions of photosynthesis from the light reactions. Although these two partial processes were closely interrelated, and known to depend on each other, from about 1940 they were no longer studied as a single unit.

Finally, it was repeatedly emphasised in this study that the photosynthesis researchers usually pursued a range of different modelling options concurrently, all the while keeping an eye on the collective at large. They resorted to this *pluralism of alternatives* chiefly because it was so difficult to pinpoint the exact solution to the main problem photosynthesis, in what was, without question, an extremely uncertain field of research. Indeed, no one model was ever taken to be the definite solution. There were always several alternatives being developed at the same time, even those that did not appear particularly promising. As long as there was a reasonable degree of uncertainty (as was usually the case), it was worth examining most, if not all, of the possibilities. This strategy was applied to fundamentally diverging options (such as the chlorophyll complex model versus models including the photosynthetic unit concept) as well as to "local" alternatives (such as whether 2-phosphoglyceric acid [2-PGA] or 3-PGA was the first product of thermochemical carbon dioxide reduction). Incidentally, the implication of this strategy was that at no point was there one "winning" model, not even in 1960; there were always families of model variants being discussed. The debate about the origin of photosynthetic oxygen evolution provides a fine example of the persistent pursuit of alternatives. From the early nineteenth century and well into the 1930s, the standard notion was that the oxygen released in photosynthesis originated from the carbon dioxide. However, researchers also explored the possibility that the oxygen might, in fact, originate from the water. Not many scientists had much faith in the latter hypothesis but it was still a viable option that they could not afford to ignore. And even though it ended up being the correct hypothesis, it was entirely unforeseeable, and the possibility was never excluded that some of the oxygen might still result directly from carbon dioxide reduction.

The need to reconsider a model arose whenever experimental results seemed to contradict the body of established knowledge – if either the model in its current state could not explain the data or if it was felt that the data were at variance with some of the consequences of the model. (Theoretical developments could also initiate a process of reconsideration, although this was far less frequently the case in photosynthesis research.) Researchers could *modify their causal graphs* in a number of different ways: add new cofactors or even completely new modules; construe alternative pathways to produce an effect or insert intermediate processes that had so far been neglected; redefine some of the factors or alter the composition of certain bundles, and so on. For the most part, it was not immediately apparent which of

these options would lead to the solution – and, hence, there were surprisingly many defenders, albeit temporary, of the existing alternatives. (In this respect, too, labour division within the collective worked smoothly.)

Recall, for example, Robert Emerson and William Arnold's 1932 finding that in photosynthesis only one molecule of oxygen was produced per couple of thousand molecules of chlorophyll. At the time, the standard model included the assumption that oxygen was produced by the direct interaction of chlorophyll molecules with molecules of carbon dioxide, in a 1:1 relationship, which was clearly in conflict with the new data. The collective reacted in a number of ways. At first, most of the scientists seriously doubted the validity of the data; James Franck, for example, was convinced for a long time that Emerson and Arnold had not stimulated the system to operate at its maximum efficiency. Others, including Emerson himself, tried to account for the data by introducing new factors to the standard model – such as the suggestion that an enzyme that was only present in very low concentrations might be involved in oxygen evolution. And a third group, notably headed by Hans Gaffron and Kurt Wohl, postulated that fundamental changes concerning the action of chlorophyll be made to the standard model; they proposed that, instead of one chlorophyll molecule acting on one molecule of carbon dioxide, thousands of light-absorbing molecules might be "cooperating" in photosynthesis. All these alternatives were pursued, although Franck's and Emerson's more conservative approaches were, at first, strongly favoured. Gaffron and Wohl's modification implied that a previously unheard-of mechanism operated in photosynthesis; and, although the assumption would have explained the data, most scientists considered that their idea was purely speculative and unacceptable, particularly given the fact that Gaffron and Wohl had failed to give any detailed demonstration of how this cooperative mechanism might function. The situation only changed when the concept of energy resonance transfer was brought up and applied to photosynthesis studies.

This reluctance on the part of researchers to revise or drop long-held model assumptions is widespread. As a rule, scientists tend to respond to new data or revised theoretical knowledge by trying to find ways to modify and expand the existing models as moderately as possible. It is only when these efforts constantly fail and alternative and more promising approaches materialise that the collective "abandons" certain models. This "dropping" of a model variant is, more often than not, a rather unspectacular event: nobody stops to falsify a hypothesis in public. For example, no one stood up to announce the end of the two-carboxylation models or the spectacular thioctic acid hypothesis supported so strongly by Melvin Calvin. If, as a result of new empirical evidence, a model cannot be fruitfully modified, the pertinent model families simply fade out of the picture. This was the case, for example, with the chlorophyll-complex model. A sound methodological foundation lies behind this unpretentious abandoning of models. Take the maximum quantum yield controversy: neither Emerson nor anyone else was able to *falsify* Warburg's results on the maximum quantum yield, as it was impossible to demonstrate the causal *irrelevance* of the eccentric experimental conditions that Warburg and Dean Burk had advanced. It is plainly impossible to falsify causal hypotheses; alternative pathways, incomplete bundles, imperfectly realised experimental conditions, and

so on, can always have an effect. All Emerson could do (and did, as far as he was able) was to demonstrate the *relevance* of certain conditions for certain results and then draw inferences as to why these results did not reflect the maximum quantum yield of actual photosynthesis. If this proves impossible, if no relevant factors can be found (as in the search for formaldehyde), the only option open to researchers is to wait until either the question has lost its relevance or new methods have emerged, such as the radioactive tracer technique that Kamen and Ruben used to look for formaldehyde, before they definitively dropped this idea in favour of better alternatives.

Having so far reflected on the epistemological side of the modelling process, it needs to be underlined that making causal inferences is an enterprise that hinges on the mastering of experimental practice: this can clearly be demonstrated by the fact that photosynthesis researchers spent so much time and energy on algae culturing. The more experiments they carried out using algal cells, the more intricate details they discovered about the complex metabolic reactions of these organisms. The physiological state of the algae turned out to be one of the most decisive influencing factors on the cells' photosynthetic performance. From the 1930s onwards, most researchers chose to work with a standard strain of *Chlorella*, which Emerson had originally introduced to his studies; and the exchange of information on model organisms and recipes for culturing media made up a large part of the correspondence of the actors. It is very likely that chemists such as Calvin, biophysicists such as Louis N. M. Duysens and even plant physiologists of later generations were no longer familiar with the reasons for the choice of species (*Chlorella* or *Scenedesmus*) or the algal cultivation techniques. They continued with established tradition, not only because they trusted their predecessors' competencies but also to ensure that their results could be compared with earlier findings. In this sense, model organisms such as *Chlorella* really did "incorporate" experimental knowledge: they were needed to satisfy the homogeneity condition, although, perhaps, nobody knew exactly which of their many properties were causally influential. The same is true of other aspects of experimental practice. The importance of tacit knowledge is impressively demonstrated by the fact that the actors often sought to solve controversies by conducting experiments together: there might always be aspects of the know-how of an experimenter, which are causally relevant to the outcome of the experiment, yet so self-evident to the experimenter herself that it does not even occur to her to explicate these details – unless, of course, a controversy arises.

The difficulties of experimental practice and the need to master the pertinent methods are two of the main reasons why scientists are usually so conservative, not only in their support of model hypotheses but also in terms of research techniques and explanatory approaches. Warburg as well as many others investigated *Chlorella* cells in certain media manometrically for most of their working lives. The development of techniques and systems, as well as the recognition of their possibilities and their shortcomings, were undeniably central elements in the process of modelling photosynthesis. But they were not the driving forces. Manometry established itself as the domineering technique in the field not because of the fact that it had many unexplored surprises to offer but because Warburg had persuasively demonstrated in the years around 1920 that some of the causally relevant factors

in photosynthesis could be successfully investigated using this method, more so than by the application of other techniques. Searching for kinetic information by manometrically measuring the process soon replaced the mainly stoichiometrically guided hunt for chemical intermediates. Once the choice of technique had been made, researchers then kept to it so as to be able to compare experimental outcomes. Undoubtedly, methods do guide research decisions to a surprising extent. That researchers jumped from one theme or field to another, as Warburg did, was clearly prompted by the strategy to exploit the technique's possibilities in as many disciplines as possible. However, the specific way in which Warburg applied manometry to photosynthesis was not inherently determined by the method itself but was rather motivated by the desire to solve a problem on the basis of individual competencies.

This even holds when individuals put their faith in the wrong hypothesis; they might err or overlook confounding factors in their experimental set-up. Although the story of modelling the mechanism of photosynthesis ended, eventually, in success, it is also evident that the modelling process did not proceed in a linear manner. Although there were almost no instances of epistemically unguided experimental tinkering in the work of individual scientists, there were unquestionably examples of unexpected discoveries – for example, when Emerson set out to investigate the influence of blue light on the gas exchange of *Chlorella* cells and discovered instead the Enhancement Effect on photosynthetic efficiency. Some phases progressed slowly – existing model alternatives were continuously worked on, without the scientists having any idea as to where the path would eventually lead. There were other phases in which the researchers failed to digest the new data, such as when certain findings in the 1930s indicated the existence of a hitherto unheard-of "photosynthetic unit". And there were instances of explanatory breakthroughs that did not add anything to the existing body of factual knowledge. The suggestion of the first Z-scheme by Robin Hill and Fay Bendall could be regarded as falling into this category. The process was not sequential, and it was certainly not foreseeable, let alone inevitable. However, it can still be regarded, if only retrospectively as the result of the goal-oriented action undertaken by a number of scientists, who, guided by well-proven heuristics and methodical knowledge, influenced by their individual disciplinary backgrounds and helped along with an extra portion of good luck, wanted to contribute collectively to solving the intricate details of the complex mechanism of photosynthesis.

Bibliography

Allen, G. E. (1975), *Life Science in the Twentieth Century*, Cambridge History of Science, Cambridge University Press, Cambridge etc.

Allen, M. B., Arnon, D. I., Capindale, J. B., Whatley, F. R. & Durham, L. J. (1955), 'Photosynthesis by isolated chloroplasts III. Evidence for complete photosynthesis', *Journal of the American Chemical Society* **77**, 4149–4155.

Allen, M. B., Whatley, F. R. & Arnon, D. I. (1958), 'Photosynthesis by isolated chloroplasts: VI. Rates of conversion of light into chemical energy in photosynthetic phosphorylation', *Biochim. Biophys. Acta* **27**, 16–23.

Allen, M. B., Whatley, F. R., Rosenberg, L. L., Capindale, J. B. & Arnon, D. I. (1957), Photosynthetic Carbon Dioxide Fixation by Broken Chloroplasts, *in* H. Gaffron, A. H. Brown, F. C. Stacy, R. Livingston, E. I. Rabinowitch, B. L. Strehler & N. E. Tolbert, Ed., 'Research in Photosynthesis: Papers and Discussions Presented at the Gatlinburg Conference, October 25-29, 1955', Interscience Publishers, S. 288–295.

Amesz, J. (1998), The Two Photosystems of Photosynthesis, *in* S.-D. Kung & S.-F. Yang, Ed., 'Discoveries in Plant Biology', Bd. 2, World Scientific Publishing, Singapore etc., S. 1–13.

Anonymous (1961), 'Grünes Geheimnis', *Der Spiegel* **48**, 88–89.

Arnold, W. (1949), A Calorimetric Determination of the Quantum Yield in Photosynthesis, *in* J. Franck & W. E. Loomis, Ed., 'Photosynthesis in Plants', Iowa State College Press, Ames, Iowa, S. 273–276.

Arnold, W. (1991), 'Experiments', *Photosynthesis Research* **27**, 73–82.

Arnold, W. & Kohn, H. I. (1934), 'The chlorophyll unit in photosynthesis', *Journal of General Physiology* **18**, 109–112.

Arnold, W. & Oppenheimer, J. R. (1950), 'Internal conversion in the photosynthetic mechanism of blue-green algae', *Journal of General Physiology* **133**, 423–435.

Arnold, W. A. (1960), Life: A Collection of Probable or Improbable Events, *in* L. G. Augenstine, Ed., 'Bioenergetics. Considerations of Processes of Absorption, Stabilization, Transfer, and Utilization (Proceedings of a Symposium [...] at the Brookhaven National Laboratory, October 12-16, 1959', Academic Press, New York / London, S. 324–325.

Arnon, D. I. (1951), 'Extracellular photosynthetic reactions', *Nature* **167**, 1008–1010.

Arnon, D. I. (1954*a*), 'Photosynthesis by isolated chloroplasts', *Nature* **174**, 394–401.

Arnon, D. I. (1954*b*), 'Photosynthesis by isolated chloroplasts II. Photosynthetic phosphorylation, the conversion of light into phosphate bond energy', *Journal of the American Chemical Society* **74**, 6324–6329.

Arnon, D. I. (1955), 'The chloroplast as a complete photosynthetic unit', *Science* **122**, 9–16.

Arnon, D. I. (1956), Localization of Photosynthesis in Chloroplasts, *in* O. H. Gaebler, Ed., 'Enzymes: Units of Biological Structure and Function', Academic Press, New York, S. 279–313.

Arnon, D. I. (1959), 'Conversion of light into chemical energy in photosynthesis', *Nature* **184**, 10–21.

Arnon, D. I. (1977), Photosynthesis 1950-1975: Changing Concepts and Perspectives, *in* A. Trebst & M. Avron, Ed., 'Photosynthesis I: Photosynthetic Electron Transport and Photophosphorylation', Springer, Berlin / Heidelberg, S. 7–56.

Arnon, D. I. (1984), 'The discovery of photosynthetic phosphorylation', *Trends in Biochemical Sciences* **9**, 258–262.

Arnon, D. I. (1987), 'Photosynthetic CO_2 assimilation by chloroplasts: Assertion, refutation, discovery', *Trends in Biochemical Sciences* **12**, 39–42.

Arnon, D. I. (1988), 'The discovery of ferredoxin: the photosynthetic path', *Trends in Biochemical Sciences* **13**, 30–33.

Arnon, D. I., Allen, M. B. & Whatley, F. R. (1956), 'Photosynthesis by isolated chloroplasts IV. General concept and comparison of three photochemical reactions', *Biochim. Biophys. Acta* **20**, 449–461.

Arnon, D. I., Whatley, F. R. & Allen, M. B. (1957), 'Triphosphopyridine nucleotide as a catalyst of photosynthetic phosphorylation by chloroplasts', *Nature* **180**, 182–185.

Arnon, D. I., Whatley, F. R. & Allen, M. B. (1958), 'Assimilatory power in photosynthesis', *Science* **127**, 1026–1034.

Aronoff, S. & Calvin, M. (1948), 'Phosphorus turnover and photosynthesis', *Plant Physiology* **23**, 351–358.

Avron, M. & Jagendorf, A. T. (1957), 'Some further investigations on chloroplast TPNH diaphorase', *Archiv. Biochem. Biophys.* **72**, 17–24.

Axelrod, B., Bandurski, R. S., Greiner, C. M. & Jang, R. (1953), 'The metabolism of hexose and pentose phosphates in higher plants', *The Journal of Biological Chemistry* **202**, 619–634.

Badin, E. J. & Calvin, M. (1950), 'The path of carbon in photosynthesis. IX. Photosynthesis, photoreduction and the hydrogen-oxygen-carbon dioxide dark reaction', *Journal of the American Chemical Society* **72**, 5266–5270.

Baeyer, A. J. F. v. (1870), 'Über die Wasserentziehung und ihre Bedeutung für das Pflanzenleben und die Gährung', *Berichte der Deutschen Chemischen Gesellschaft zu Berlin* **3**, 63–75.

Bailer-Jones, D. (2000), 'Modelling extended extragalactic radio sources', *Studies in History and Philosophy of Modern Physics* **31**, 49–74.

Bailer-Jones, D. (2009), *Scientific Models in Philosophy of Science*, Univ. of Pittsburgh Press.

Ball, E. G. (1942), Oxidative Mechanisms in Animal Tissues, *in* 'A Symposium on Respiratory Enzymes', Univ. of Wisconsin Press, Madison, S. 16–32.

Bannister, T. T. (1972), 'The careers and contributions of Eugene Rabinowitch', *Biophysical Journal* **12**, 707–718.

Barker, H. A. (1982), Radioactive Carbon in Bacterial Metabolism: Something Old and Something New, *in* N. O. Kaplan & A. Robinson, Ed., 'From Cyclotrons to Cytochromes: Essays in Molecular Biology and Chemistry', Academic Press, New York, S. 67–75.

Barker, H. A. & Hungate, R. E. (1990), 'Cornelis Bernardus Van Niel (1897-1985)', *Biographical Memoirs of the National Academy of Sciences* **59**, 389–423.

Bassham, J. A. (1997), 'Melvin Calvin (1911-1997)', *Photochemistry and Photobiology* **65**, 605–606.

Bassham, J. A. (2003), 'Mapping the carbon reduction cycle: a personal retrospective', *Photosynthesis Research* **76**, 35–52.

Bassham, J. A. & Calvin, M. (1960), The Path of Carbon in Photosynthesis, *in* A. Pirson, Ed., 'Handbuch der Pflanzenphysiologie', Springer, Berlin/ Göttingen/ Heidelberg, S. 884–922.

Bassham, J. A., Benson, A. A. & Calvin, M. (1950), 'The path of carbon in photosynthesis VIII. The rôle of malic acid', *Journal of Biological Chemistry* **185**, 781–787.

Bassham, J. A., Benson, A. A., Kay, L. D., Harris, A. Z., Wilson, A. T. & Calvin, M. (1954), 'The path of carbon in photosynthesis. XXI. The cyclic regeneration of carbon dioxide acceptor', *Journal of the American Chemical Society* **76**, 1760–1770.

Baumgartner, M. & Grasshoff, G. (2004), *Kausalität und kausales Schliessen. Eine Einführung mit interaktiven Übungen*, Bern Studies in the History and Philosophy of Science, Bern.

Baur, E. (1913), 'Über die Genesis der Kohlenhydrate', *Naturwissenschaften* **1**(20), 474–477.

Bay, Z. & Pearlstein, R. M. (1963), 'A theory of energy transfer in the photosynthetic unit', *Proceedings of the National Academy of Sciences (USA)* **50**, 1071–1078.

Bechtel, W. (2006), *Discovering Cell Mechanisms: The Creation of Modern Cell Biology*, Cambridge University Press.

Bechtel, W. & Abrahamsen, A. (2005), 'Explanation: a mechanist alternative', *Studies in History and Philosophy of Biological and Biomedical Sciences* **36**, 421–441.

Beckel, A. L. (1987), 'Breaking New Waters. A Century of Limnology at the University of Wisconsin', *Transactions of the Wisconsin Academy of Sciences (Special Issue)*.

Bendall, D. S. (1994), 'Robert Hill', *Biographical Memoirs of Fellows of the Royal Society* **40**, 141–171.

Bendall, D. S. (2004), 'The unfinished story of cytochrome f', *Photosynthesis Research* **80**, 265–276.

Bendall, D. S. & Walker, D. A. (1991), 'Obituary: Robert (Robin) Hill (1899-1991)', *Photosynthesis Research* **30**, 1–5.

Benson, A. A. (1951), 'Identification of ribulose in $^{14}CO_2$ photosynthesis products', *Journal of the American Chemical Society* **73**, 2971–2972.

Benson, A. A. (1982), Tracers in Biology, *in* N. O. Kaplan & A. Robinson, Ed., 'From Cyclotrons to Cytochromes: Essays in Molecular Biology and Chemistry', Academic Press, New York, S. 59–65.

Benson, A. A. (1995), 'Saga of a great theory of photosynthesis', *Am. Soc. Plant Physiol. Newsletter* **22**, 5–6.

Benson, A. A. (2002*a*), 'Following the path of carbon in photosynthesis: a personal story', *Photosynthesis Research* **73**, 29–49.

Benson, A. A. (2002*b*), 'Paving the path', *Annual Review of Plant Biology* **53**, 1–25.

Benson, A. A. & Calvin, M. (1947), 'The dark reductions of photosynthesis', *Science* **105**, 648–649.

Benson, A. A. & Calvin, M. (1950), 'Carbon dioxide fixation by green plants', *Annual Review of Plant Physiology* **1**, 25–42.

Benson, A. A., Bassham, J. A. & Calvin, M. (1951), 'Sedoheptulose in photosynthesis by plants', *Journal of the American Chemical Society* **73**, 2970.

Benson, A. A., Bassham, J. A., Calvin, M., Goodale, T. C., Haas, V. A. & Stepka, W. (1950), 'The path of carbon in photosynthesis. V. Paper chromatography and radioautography of the products', *Journal of the American Chemical Society* **72**, 1710–1718.

Benson, A. A., Bassham, J. A., Calvin, M., Hall, A. G., Hirsch, H. E., Kawaguchi, S., Lynch, V. & Tolbert, N. E. (1952*a*), 'The path of carbon in photosynthesis. XV. Ribulose and sedoheptulose', *Journal of Biological Chemistry* **196**, 703–716.

Benson, A. A., Calvin, M., Haas, V. A., Aronoff, S., Hall, A. G., Bassham, J. A. & Weigl, J. W. (1949), C^{14} in Photosynthesis, *in* J. Franck & W. E. Loomis, Ed., 'Photosynthesis in Plants', State College Press, Ames, Iowa, S. 381–401.

Benson, A. A., Kawaguchi, S., Hayes, P. & Calvin, M. (1952*b*), 'The path of carbon in photosynthesis. XVI. Kinetic relationships of the intermediates in steady state photosynthesis', *Journal of the American Chemical Society* **74**, 4477–4482.

Bergia, S. & Navarro, L. (1988), 'Recurrences and continuity in Einstein's research on radiation between 1905 and 1916', *Archive for History of Exact Sciences* **38**, 79–99.

Besse, I. & Buchanan, R. B. (1998), Plant Ferredoxin: a Multifunctional Electron Carrier Protein With an Unusual Genealogy, *in* S.-D. Kung & S.-F. Yang, Ed., 'Discoveries in Plant Biology', Bd. 2, World Scientific Publishing, Singapore etc., S. 137–156.

Beyerchen, A. D. (1996), Emigration from Country and Discipline: The Journey of a German Physicist into American Photosynthesis Research, *in* M. G. Ash & A. Söllner, Ed., 'Forced Migration and Scientific Change', Cambridge University Press, Cambridge, Mass., S. 71–85.

Bhagvat, K. & Hill, R. (1951), 'Cytochrome oxidase in higher plants', *New Phytologist* **50**, 112–120.

Bishop, N. I. (1958), 'The influence of the herbicide, DCMU, on the oxygen-evolving system of photosynthesis', *Biochim. Biophys. Acta* **27**, 205–206.

Bishop, N. I. (1959), 'The reactivity of a naturally occurring quinone (Q-255) in photochemical reactions of isolated chloroplasts', *Proceedings of the National Academy of Sciences (USA)* **45**, 1696–1702.

Black, C. C. & Mayne, B. C. (2006), 'Allan H. Brown (1917-2004), editor and educator: A career of fascination with the biological roles of O_2 in terrestrial life and possibly in extraterestrial life', *Photosynthesis Research* **87**, 159–163.

Black, M. (1962), *Models and Metaphors: Studies in Language and Philosophy*, Cornell University Press, Ithaca.

Blackman, F. F. (1905), 'Optima and limiting factors', *Annals of Botany* **19**, 281–295.

Blankenship, R., Amesz, J., Holten, D. & Jortner, J. (1989), 'Tunneling Processes in Photosynthesis (Special issue dedicated to Don DeVault)', Photosynthesis Research 22 (1).

Blinks, L. R. (1957), Chromatic Transients in Photosynthesis of Red Algae, *in* H. Gaffron, A. H. Brown, C. S. French, R. Livingston, E. I. Rabinowitch, B. L. Strehler & N. E. Tolbert, Ed., 'Research in Photosynthesis', Interscience Publishers, New York, S. 444–449.

Blinks, L. R. (1960*a*), 'Action spectra of chromatic transients and the Emerson effect in marine algae', *Proceedings of the National Academy of Sciences (USA)* **46**, 327–333.

Blinks, L. R. (1960*b*), Chromatic Transients in the Photosynthesis of Green, Brown, and Red Algae, *in* M. B. Allen, Ed., 'Comparative Biochemistry of Photoreactive Systems', Academic Press, New York, S. 329–341.

Blinks, L. R. (1960*c*), 'Relation of photosynthetic transients to respiration', *Science* **131**, 1316.

Blinks, L. R. (1974), 'Winthrop John Vanleuven Osterhout (1871-1964)', *Biographical Memoirs of the National Academy of Sciences* **44**, 213–249.

Boberlin, U. (1993), *Photochemische Untersuchungen von R. Bunsen und H. Roscoe im Vergleich mit den Arbeiten J. W. Drapers und W. C. Wittwers: Die Anfänge der quantitativen Photochemie im 19. Jahrhundert*, Köster, Berlin.

Bogen, J. (2008), 'Causally Productive Activities.', *Studies in History and Philosophy of Science, Part A* **39**, 112–123.

Boorse, H. A., Motz, L. & Weaver, J. H. (1989), *The Atomic Scientists: A Biographical History*, John Wiley.

Borsook, H. (1978), 'Interview by Mary Terrall. Pasadena, California, April 5, 1978', Oral History Project, California Institute of Technology Archives. Retrieved September 2008 from the World Wide Web: http://resolver.caltech.edu/CaltechOH:OH_Borsook_H.

Brackett, F. S. (1953), 'Time course and quantum efficiency of photosynthesis in Chlorella', *Journal of General Physiology* **36**, 563–579.

Bredig, G. (1914*a*), 'Katalytische Ameisensäuresynthese unter Druck und ein photochemisch-katalytisches Assimilationsmodell. Vortrag', *Chemiker-Ztg.* (57), 186–187.

Bredig, G. (1914*b*), 'Welche Zwischenprodukte entstehen bei der Assimilation der Kohlensäure durch die Pflanze?', *Die Umschau* **18**, 362–364.

Bredig, G. (1915), 'Gleichgewicht der Kohlensäurereduktion. Vortrag', *Chemiker-Ztg.* **12**(72), 72–73.

Brock, W. H. (1997), *Justus von Liebig : the Chemical Gatekeeper*, Cambridge University Press, Cambridge etc.

Brodhun, E. (1913), 'Die Physikalisch-Technische Reichsanstalt. Fünfundzwanzig Jahre ihrer Tätigkeit', *Naturwissenschaften* **1**(14), 321–325.

Brody, S. S. (1995), 'We remember Eugene', *Photosynthesis Research* **43**, 67–74.

Brody, S. S. (2002), 'Fluorescence lifetime, yield, energy transfer and spectrum in photosynthesis, 1950-1960', *Photosynthesis Research* **73**, 127–132.

Brody, S. S. & Rabinowitch, E. I. (1957), 'Excitation lifetime of photosynthetic pigments in vitro and in vivo', *Science* **125**, 555.

Brown, A. H. (1953), 'The effects of light on respiration, using isotopically enriched oxygen', *American Journal of Botany* **40**, 719–729.

Brown, A. H. & Frenkel, A. W. (1953), 'Photosynthesis', *Annual Review of Biochemistry* **22**, 423–458.

Brown, A. H. & Good, N. (1955), 'Photochemical reduction of oxygen in chloroplast preparations and in green plant cells. I. The study of oxygen exchanges *in vitro* and *in vivo*', *Archives of Biochemistry and Biophysics* **57**, 340–354.

Brown, A. H., Fager, E. W. & Gaffron, H. (1949), Kinetics of a Photochemical Intermediate in Photosynthesis, *in* J. Franck & W. E. Loomis, Ed., 'Photosynthesis in Plants', Iowa State College Press, S. 403–422.

Brown, H. T. (1905), 'The reception and utilisation of energy by a green leaf', *Nature* **71**(March 30), 522–526.

Brown, H. T. & Escombe, F. (1900), 'Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants', *Philosophical Transactions, Series B* **193**, 223–291.

Brown, H. T. & Escombe, F. (1905), 'Researches on some of the physiological processes of green leaves, with special reference to the interchange of energy between the leaf and its surroundings', *Proceedings of the Royal Society of London, Series B* **76**(507), 29–111.

Buchanan, R. B. (1995), 'The life of Daniel I. Arnon', *Photosynthesis Research* **46**, 3–6.

Buchanan, R. B. (2001), 'Daniel I. Arnon (1910-1994)', *Biographical Memoirs of Fellows of the Royal Society* **80**, 2–3.

Buchanan, R. B. & Arnon, D. I. (1990), 'A reverse Krebs cycle in photosynthesis: consensus at last', *Photosynthesis Research* **24**, 47–53.

Buchanan, R. B., Douce, R. & Lichtenthaler, H. K. (2007), 'Andrew A. Benson', *Photosynthesis Research* **92**, 143–144.

Burk, D., Cornfield, J. & Schwartz, M. (1951), 'The efficient transformation of light into chemical energy in photosynthesis', *The Scientific Monthly* **73**, 213–223.

Burk, D., Hendricks, S., Korzenovsky, M., Schocken, V. & Warburg, O. (1949), 'The maximum efficiency of photosynthesis: A rediscovery', *Science* **110**, 225–229.

Butlerov, A. M. (1861), 'Bildung einer zuckerartigen Substanz durch Synthese', *Annalen der Chemie und Pharmazie* **120**, 295–298.

Butler, W. L. (1962), 'Effects of red and far-red light on the fluorescence yield of chlorophyll *in vivo*', *Biochim. Biophys. Acta* **64**, 309–317.

Cahan, D. (1989), *An Institute for an Empire: The Physikalisch-Technische Reichsanstalt, 1871-1918*, Cambridge University Press, New York.

Calvin, M. (1962), 'The path of carbon in photosynthesis', *Science* **135**, 879–889.

Calvin, M. (1964), The Path of Carbon in Photosynthesis, *in* 'Nobel Lectures, Chemistry 1942-1962', Elsevier Publishing Company, Amsterdam, S. 618–644.

Calvin, M. (1989), 'Forty years of photosynthesis and related activities', *Photosynthesis Research* **21**, 3–16.

Calvin, M. (1992), *Following the Trail of Light. A Scientific Odyssey*, American Chemical Society, Washington, D.C.

Calvin, M. & Benson, A. A. (1948), 'The path of carbon in photosynthesis', *Science* **107**, 476–480.

Calvin, M. & Massini, P. (1952), 'The path of carbon in photosynthesis. XX: The steady state', *Experientia* **8**, 445–457.

Caneva, K. L. (1993), *Robert Mayer and the Conservation of Energy*, Princeton University Press, Princeton.

Carson, S. F., Foster, J. W., Ruben, S. & Barker, H. A. (1941), 'Radioactive Carbon as an Indicator of Carbon Dioxide Utilization. V. Studies on the Propionic Acid Bacteria', *Proceedings of the National Academy of Sciences (USA)* **27**, 229–235.

Chance, B. (1983), 'Commentary on the paper Chance & Wlliams (1956)', *Citation Classics Commentaries* **49**, 232 (December 5). URL: http://www.garfield.library.upenn.edu/classics1983/A1983RQ98000001.pdf.

Chance, B. (2004), 'The stopped-flow method and chemical intermediates in enzyme reactions – a personal essay', *Photosynthesis Research* **80**, 387–400.

Chance, B. & Williams, G. R. (1955), 'Respiratory enzymes in oxidative phosphorylation. I-IV. J. Biol. Chem. 217:, 1955.', *Journal of Biological Chemistry* **217**, 383–393; 395–407; 409–427; 429–438.

Chance, B. & Williams, G. R. (1956), 'The respiratory chain and oxidative phosphorylation', *Advances in Enzymology* **17**, 65–134.

Chance, B., Williams, G. R., Holmes, W. F. & Higgins, J. (1955), 'Respiratory enzymes in oxidative phosphorylation. V.', *Journal of Biological Chemistry* **217**, 439–451.

Chung, K.-T. & Ferris, D. H. (1996), 'Martinus Willem Beijerinck (1851-1931): Pioneer of general microbiology', *AMS News* **62**, 539–543.

Churchland, P. M. (1970), 'The logical character of action explanations', *Philosophical Review* **79**, 214–236.

Clayton, R. K. (2002), 'Research on photosynthetic reaction centers from 1932 to 1987', *Photosynthesis Research* **73**, 63–71.

Consden, R., Gordon, A. H. & Martin, A. J. P. (1944), 'Qualitative analysis of proteins: A partition chromatographic method using paper', *Biochemical Journal* **38**, 224–232.

Craver, C. F. (2006), 'When mechanistic models explain', *Synthese* **153**, 355–376.

Creager, A. N. H., Lunbeck, E. & Wise, M. N., Ed. (2007), *Science Without Laws: Model Systems, Cases, Exemplary Narratives*, Duke University Press.

Czapek, F. (1913), *Biochemie der Pflanzen*, 2., revised Aufl., Fischer, Jena.

Darden, L. (2002), 'Strategies for discovering mechanisms: Schema instantiation, modular subassembly, forward/backward chaining', *Philosophy of Science* **69**, S354–S365.

Darden, L. (2008), 'Thinking again about biological mechanisms', *Philosophy of Science* **75**, 958–969.

Davenport, H. E. (1952), 'Cytochrome components in chloroplasts', *Nature* **170**, 1112–1114.

Davenport, H. E. (1959), 'Coenzyme reduction by illuminated chloroplasts', *Biochemical Journal* **73**, 45p (Proceedings of the Biochemical Society).

Davenport, H. E. (1960), 'A protein from leaves catalysing the reduction of metmyoglobin and triphosphopyridine nucleotide by illuminated chloroplasts', *Biochemical Journal* **77**, 471–477.

Davenport, H. E. & Hill, R. (1952), 'The preparation and some properties of cytochrome *f*', *Proceedings of the Royal Society of London, Series B* **139**, 327–345.

Davenport, H. E. & Hill, R. (1960), 'A protein from leaves catalysing the reduction of haem-protein compounds by illuminated chloroplasts', *Biochemical Journal* **74**, 493–501.

Davenport, H. E., Hill, R. & Whatley, F. R. (1952), 'A natural factor catalysing reduction of methaemoglobin by isolated chloroplasts', *Proceedings of the Royal Society of London, Series B* **139**, 346–358.

Deichmann, U. (2001*a*), The Expulsion of German-Jewish Chemists and Biochemists and their Correspondence with Colleagues in Germany after 1945: The Impossibility of Normalization?, *in* M. Szöllösi-Janze, Ed., 'Science in the Third Reich', Berg, Oxford / New York, S. 243–280.

Deichmann, U. (2001*b*), *Flüchten, Mitmachen, Vergessen. Chemiker und Biochemiker in der NS Zeit*, Wiley VCH.

Demeter, S. & Govindjee (1989), 'Thermoluminescence in plants', *Physiologia Plantarum* **75**, 121–130.

Dent, C. E., Fink, K. & Fink, R. M. (1947*a*), 'Application of filter paper partition chromatography to radioactive tracer studies', *Nature* **160**, 801–803.

Dent, C. E., Stepka, W. & Steward, F. C. (1947*b*), 'Detection of free amino acids of plant cells by partition chromatography', *Nature* **160**, 682–683.

DeVault, D. & Chance, C. (1966), 'Studies of photosynthesis using a pulsed laser. I. Temperature dependence of cytochrome oxidation rate in chromatium. Evidence for tunneling', *Biophysical Journal* **6**, 825–847.

DeVault, D., Parkes, J. H. & Chance, B. (1967), 'Electron tunnelling in cytochromes', *Nature* **215**, 642–644.

Dmetrievsky, O. D., Ermolaev, V. L. & N., T. A. (1957), 'Direct measurement of the lifetimes of chlorophyll and analogous pigments in various media', *Doklady Akademii Nauk* **114**, 468–470.

Dole, M. & Jenks, G. (1944), 'Isotopic composition of photosynthetic oxygen', *Science* **100**, 409.

Dorner, R. W., Kahn, A. & Wildman, S. G. (1957), 'The proteins of green leaves. VII. Synthesis and decay of the cytoplasmic proteins during the life of a tobacco leaf', *Journal of Biological Chemistry* **229**, 945–952.

Dutton, H. J. (1997), 'Carotenoid-sensitized photosynthesis: Quantum efficiency, fluorescence and energy transfer', *Photosynthesis Research* **52**, 175–185.

Dutton, H. J. & Manning, W. M. (1941), 'Evidence for carotenoid-sensitized photosynthesis in the diatom *Nitzschia closterium*', *American Journal of Botany* **28**, 516–526.

Dutton, H. J., Manning, W. M. & Duggar, B. M. (1943), 'Chlorophyll fluorescence and energy transfer in diatom *Nitzschia closterium*', *Journal of Physical Chemistry* **47**, 308–313.

Duysens, L. N. M. (1951), 'Transfer of light energy within the pigment systems present in photosynthesizing cells', *Nature* **168**, 548–550.

Duysens, L. N. M. (1952), Transfer of excitation energy in photosynthesis, Ph.d., University of Utrecht, Netherlands.

Duysens, L. N. M. (1954*a*), 'Reversible changes in the absorption spectrum of Chlorella upon irradiation', *Science* **120**, 353–354.

Duysens, L. N. M. (1954*b*), 'Reversible photo-oxidation of a cytochrome pigment in photosynthesizing Rhodospirillum rubrum', *Nature* **173**, 692–693.

Duysens, L. N. M. (1955), 'Role of cytochrome and pyridine nucleotide in algal photosynthesis', *Science* **121**, 210–211.

Duysens, L. N. M. (1956), 'Energy transformations in photosynthesis', *Annual Review of Plant Physiology* **7**, 25–50.

Duysens, L. N. M. (1957), Investigations in the Photosynthetic Mechanism of Purple Bacteria by Means of Sensitive Absorption Spectrophotometry, *in* H. Gaffron, A. H. Brown, F. C. Stacy, R. Livingston, E. I. Rabinowitch, B. L. Strehler & N. E. Tolbert, Ed., 'Research in Photosynthesis: Papers and Discussions Presented at the Gatlinburg Conference, October 25-29, 1955', Interscience Publishers, S. 164–173.

Duysens, L. N. M. (1961), Cytochrome Oxidation by a Second Photochemical System in the Red Alga *Porphyridium cruentum*, *in* B. C. Christensen & B. Buchmann, Ed., 'Progress in Photobiology. Proceedings of the 3rd International Congress in Photobiology, Copenhagen, 1960', Elsevier, Amsterdam, S. 135–142.

Duysens, L. N. M. (1989), 'The discovery of the two photosynthetic systems: a personal account', *Photosynthesis Research* **21**, 61–79.

Duysens, L. N. M. & Sweers, H. E. (1963), Mechanism of Two Photochemical Reactions in Photosynthesis as Studied by Means of Fluorescence, *in* 'Studies on Microalgae and Photosynthetic Bacteria', University of Tokyo Press, S. 353–372.

Duysens, L. N. M., Amesz, J. & Kamp, B. M. (1961), 'Two photochemical systems in photosynthesis', *Nature* **190**, 510–511.

Duysens, L. N. M., Huiskamp, W. J., Vos, J. J. & van der Hart, J. M. (1956), 'Reversible changes in bacteriochlorophyll in purple bacteria upon illumination', *Biochim. Biophys. Acta* **19**, 188–190.

Edsall, J. T., Ed. (1974), *History of Bioenergetics. Special issue of the journal* Molecular and Cellular Biochemistry. *Papers from a Conference held at the American Academy of Arts and Sciences, Oct. 11-13, 1973.*

Einstein, A. (1905), 'Über einen die Erzeugung und Verwandlung des Lichtes betreffenden heuristischen Gesichtspunkt', *Annalen der Physik* **17**, 132–148.

Einstein, A. (1909), 'Über die Entwickelung unserer Anschauungen über das Wesen und die Konstitution der Strahlung', *Verhandlungen der Deutschen Physikalischen Gesellschaft* **11**, 482–500.

Einstein, A. (1912*a*), 'Nachtrag zu meiner Arbeit: "Thermodynamische Begründung des photochemischen Aequivalentgesetzes"', *Annalen der Physik* **38**, 881–884.

Einstein, A. (1912*b*), 'Thermodynamische Begründung des photochemischen Aequivalentgesetzes', *Annalen der Physik* **37**, 832–838.

Einstein, A. (1913), 'Déduction thermodynamique de la loi de l'équivalence photochimique', *Journal de Physique* **3**, 277–282.

Emerson, R. (1927), 'The effect of certain respiratory inhibitors on the respiration of Chlorella', *Journal of General Physiology* **10**, 469–477.

Emerson, R. (1936), 'A review of recent investigations in the field of chlorophyll photosynthesis', *Ergebnisse der Enzymforschung* **5**, 305–347.

Emerson, R. & Arnold, W. (1932*a*), 'The photochemical reaction in photosynthesis', *Journal of General Physiology* **16**, 191–205.

Emerson, R. & Arnold, W. (1932*b*), 'A separation of the reactions in photosynthesis by means of intermittent light', *Journal of General Physiology* **15**, 391–420.

Emerson, R. & Chalmers, R. (1955), 'Transient changes in cellular gas exchange and the problem of maximum efficiency of photosynthesis', *Plant Physiology* **30**, 504–529.

Emerson, R. & Chalmers, R. (1958), 'Speculations concerning the function and phylogenetic significance of the accessory pigments of algae', *Phycological Society of America News Bulletin* **11**, 51–56.

Emerson, R. & Green, L. (1937), 'Nature of the Blackman reaction in photosynthesis', *Plant Physiology* **12**, 537–545.

Emerson, R. & Lewis, C. M. (1939), 'Factors influencing the efficiency of photosynthesis', *American Journal of Botany* **26**(10), 808–822.

Emerson, R. & Lewis, C. M. (1941*a*), 'Carbon dioxide exchange and the measurement of the quantum yield of photosynthesis', *American Journal of Botany* **28**, 789–804.

Emerson, R. & Lewis, C. M. (1941*b*), 'The photosynthetic efficiency of phycocyanin in Chroococcus and the problem of carotenoid participation in photosynthesis', *Journal of General Physiology* **25**, 579–595.

Emerson, R. & Lewis, C. M. (1943), 'The dependence of the quantum yield of Chlorella photosynthesis on wave length of light', *American Journal of Botany* **30**, 165–178.

Emerson, R. & Rabinowitch, E. I. (1960), 'Red drop and role of auxiliary pigments in photosynthesis', *Plant Physiology* **35**, 477–485.

Emerson, R., Chalmers, R. V. & Cederstrand, C. N. (1957), 'Some factors influencing the long-wave limit of photosynthesis', *Proceedings of the National Academy of Sciences (USA)* **43**, 133–143.

Emerson, R., Stauffer, J. F. & Umbreit, W. W. (1944), 'Relationships between phosphorylation and photosynthesis in Chlorella', *American Journal of Botany* **31**, 107–120.

Engelhardt, V. A. (1930), 'Ortho- und Pyrophosphat im aeroben und anaeroben Stoffwechsel der Blutzellen', *Biochemische Zeitung* **227**, 16–38.

Engelhardt, V. A. (1939), 'Myosine and adenosinetriphosphatase', *Nature* **144**, 668–669.

Erlenmeyer, E. (1877), 'Das Wasser als Oxydations- und Reductionsmittel', *Berichte der Deutschen Chemischen Gesellschaft* **10**, 634–635.

Evans, M. C., Buchanan, R. B. & Arnon, D. I. (1966), 'A new ferredoxin-dependent carbon reduction cycle in a photosynthetic bacterium', *Proceedings of the National Academy of Sciences (USA)* **55**, 928–934.

Fager, E. W. (1949), Investigation of the Chemical Properties of Intermediates in Photosynthesis, *in* J. Franck & W. E. Loomis, Ed., 'Photosynthesis in Plants', State College Press, Ames, Iowa, S. 423–436.

Fager, E. W. & Rosenberg, J. L. (1950), 'Phosphoglyceric acid in photosynthesis', *Science* **112**, 617–618.

Fantini, B. (2000), The History of the Stazione Zoologica Anton Dohrn. An Outline, *in* 'S.Z.A.D. Napoli: Activity Report 1998/99', S. 71–105.

Fink, R. M. & Fink, K. (1948), 'Radiocarbon and filter paper chromatography', *Science* **107**, 253.

Finlay, M. R. (2009), *Growing American Rubber: Strategic Plants and the Policies of National Security.*, Rutgers University Press.

Fischer, E. (1909*a*), Synthesen in der Zuckergruppe I (Vortrag, gehalten in der Sitzung der Deutschen chemischen Gesellschaft am 23. Juni 1890), *in* 'Untersuchungen über Kohlenhydrate und Fermente', Springer, Berlin, S. 1–29. [Originally published in *Berichte der deutschen chemischen Gesellschaft* 23, 2114 (1890)].

Fischer, E. (1909*b*), Synthesen in der Zuckergruppe II, *in* 'Untersuchungen über Kohlenhydrate und Fermente', Springer, Berlin, S. 30–76. [Originally published in *Berichte der deutschen chemischen Gesellschaft* 27, 3189 (1894)].

Fleck, L. (1994), *Entstehung und Entwicklung einer wissenschaftlichen Tatsache. Einführung in die Lehre vom Denkstil und Denkkollektiv*, Suhrkamp, Frankfurt am Main.

Florkin, M. (1977), *A History of Biochemistry. Part IV. Early Studies on Biosynthesis*, Bd. 32 von *Comprehensive Biochemistry*, Elsevier, Amsterdam / Oxford / New York.

Florkin, M. (1979), *A History of Biochemistry. Part V: The Unravelling of Biosynthetic Pathways*, Bd. 33A von *Comprehensive Biochemistry*, Elsevier, Amsterdam / Oxford / New York.

Florkin, M. & Stotz, E. (1975), *A History of Biochemistry. Part III: History of the Identification of the Sources of Free Energy in Organisms.*, Bd. 31 von *Comprehensive Biochemistry*, Elsevier, Amsterdam / Oxford / New York.

Franck, J. (1925), 'Elementary processes of photochemical reactions', *Transactions of the Faraday Society* **21**, 536–542.

Franck, J. (1926), 'Emil Warburg zum achzigsten Geburtstage', *Naturwissenschaften* **14**(11), 203–207.

Franck, J. (1931), 'Emil Warburg zum Gedächtnis', *Naturwissenschaften* **19**(50), 993–997.

Franck, J. (1935*a*), 'Beitrag zum Problem der Kohlensäure-Assimilation', *Naturwissenschaften* **23**, 226–229.

Franck, J. (1935*b*), 'Remarks on photosynthesis', *Chemical Reviews* **17**, 433–438.

Franck, J. (1945), 'Photosynthetic activity of isolated chloroplasts', *Reviews of Modern Physics* **17**, 112–119.

Franck, J. (1949), 'An interpretation of the contradictory results in measurements of the photosynthetic quantum yield and related phenomena', *Archives of Biochemistry* **23**, 297–314.

Franck, J. & Gaffron, H. (1941), 'Photosynthesis, facts and interpretation', *Advances in Enzymology* **1**, 199–262.

Franck, J. & Hertz, G. (1914), 'Über Zusammenstöße zwischen Elektronen und den Molekülen des Quecksilberdampfes und die Ionisierungsspannung desselben', *Verhandlungen der Deutschen Physikalischen Gesellschaft* **16**, 457–467.

Franck, J. & Herzfeld, K. F. (1937), 'An attempted theory of photosynthesis', *Journal of Chemical Physics* **5**, 237–251.

Franck, J. & Herzfeld, K. F. (1941), 'Contributions to a theory of photosynthesis', *Journal for Physical Chemistry* **45**, 978–1025.

Franck, J. & Levi, H. (1935*a*), 'Beitrag zur Untersuchung der Fluoreszenz in Flüssigkeiten', *Zeitschrift für Physikalische Chemie* **B27**, 409–420.

Franck, J. & Levi, H. (1935*b*), 'Zum Mechanismus der Sauerstoff-Aktivierung durch fluoreszenzfähige Farbstoffe', *Naturwissenschaften* **23**, 229–230.

Franck, J. & Rabinowitch, E. I. (1934), 'Some remarks about free radicals and the photochemistry of solutions', *Transactions of the Faraday Society* **30**, 120–130.

Franck, J. & Rosenberg, J. L. (1964), 'A theory of light utilization in plant photosynthesis', *Journal of Theoretical Biology* **7**, 276–301.

Franck, J. & Teller, E. (1938), 'Migration and photochemical action of excitation energy in crystals', *Journal of Chemical Physics* **6**, 861–872.

Franck, J., French, C. S. & Puck, T. T. (1941), 'The fluorescence of chlorophyll and photosynthesis', *Journal of Physical Chemistry* **45**, 1268–1300.

French, C. S. (1937), 'The rate of CO_2 assimilation by purple bacteria at various wave lengths of light', *Journal of General Physiology* **21**, 71–87.

French, C. S. (1959), 'Robert Emerson, investigator of photosynthesis', *Science* **130**(3373 (Aug. 21)), 437–438.

French, C. S. (1961), Light, Pigments and Photosynthesis, *in* W. D. McElroy & B. Glass, Ed., 'Light and Life', The Johns Hopkins Press, Baltimore, S. 447–474.

French, C. S. (1979), 'Fifty years of photosynthesis', *Annual Review of Plant Physiology* **30**, 1–26.

French, C. S. & Anson, M. L. (1941), 'Oxygen production by isolated chloroplasts', *American Journal of Botany* **28**, 12s (abstract).

French, C. S. & Young, V. K. (1952), 'The fluorescence spectra of red algae and the transfer of energy from phycoerytherin to phycocyanin and chlorophyll', *Journal of General Physiology* **35**, 873–890.

Frenkel, A. W. (1954), 'Light-induced phosphorylation by cell-free preparations of photosynthetic bacteria', *Journal of the American Chemical Society* **76**, 5568–5569.

Frenkel, A. W. (1993), 'Reflections', *Photosynthesis Research* **35**, 103–116.

Frenkel, A. W. (1995), 'Photosynthetic phosphorylation', *Photosynthesis Research* **46**, 73–77.

Friedmann, H. C. (2004), 'From "Butyribacterium" to "E. coli": An essay on unity in biochemistry', *Perspectives in Biology and Medicine* **47**, 47–66.

Frigg, R. & Hartmann, S. (2006), Models in Science, *in* E. N. Zalta, Ed., 'The Stanford Encyclopedia of Philosophy', URL: <http://plato.stanford.edu/archives/spr2006/entries/models-science/>.

Förster, T. (1946), 'Energiewanderung und Fluoreszenz, Naturwissenschaften', **33**, 166–175.

Förster, T. (1947), 'Ein Beitrag zur Theorie der Photosynthese', *Zeitschrift für Naturforschung* **2b**, 174.

Förster, T. (1951), *Fluoreszenz organischer Verbindungen*, Vandenhoeck & Ruprecht.

Fruton, J. S. (1972), *Molecules and Life. Historical Essays on the Interplay of Chemistry and Biology*, Wiley-Interscience, New York.

Fruton, J. S. (1999), *Proteins, Enzymes, Genes: The Interplay of Chemistry and Biology*, Yale University Press, New Haven / London.

Fuller, R. C. (1999), 'Forty years of microbial photosynthesis research: where it came from and what it led to', *Photosynthesis Research* **62**, 1–29.

Gaffron, H. (1933*a*), *Biochemische Zeitschrift* **264**, 271.

Gaffron, H. (1933*b*), 'Über den Stoffwechsel der schwefelfreien Purpurbakterien', *Biochemische Zeitschrift* **260**, 1–17.

Gaffron, H. (1934), 'Über die Kohlensäure-Assimilation der roten Schwefelbakterien', *Biochemische Zeitschrift* **269**, 447–453.

Gaffron, H. (1935), 'Inwiefern ist Sauerstoff für die Kohlensäureassimilation unentbehrlich?', *Naturwissenschaften* **23**, 528–528.

Gaffron, H. (1940*a*), 'Carbon dioxide reduction with molecular hydrogen in green algae', *American Journal of Botany* **27**, 273–283.

Gaffron, H. (1940*b*), 'Studies on the induction period of photosynthesis and light respiration in green algae', *American Journal of Botany* **27**, 204–216.

Gaffron, H. (1969), 'Resistance to knowledge', *Plant Physiology* **20**, 1–40.

Gaffron, H. & Fager, E. W. (1951), 'The kinetics and chemistry of photosynthesis', *Annual Review of Plant Physiology* **2**, 87–114.

Gaffron, H. & Wohl, K. (1936), 'Zur Theorie der Assimilation', *Naturwissenschaften* **24**, 81–90; 103–107.

Gaffron, H., Brown, A. H., Stacy, F. C., Livingston, R., Rabinowitch, E. I., Strehler, B. L. & Tolbert, N. E., Ed. (1957), *Research in photosynthesis: papers and discussions presented at the Gatlinburg conference, October 25-29, 1955*, Interscience Publishers, Baltimore.

Gest, H. (2004), 'Samuel Ruben's contributions to research on photosynthesis and bacterial metabolism with radioactive carbon', *Photosynthesis Research* **80**, 77–83.

Gest, H. (2005), A personal tribute to an eminent photosynthesis researcher Martin D. Kamen (1913-2002), *in* Govindjee, J. T. Beatty, H. Gest & J. F. Allen, Ed., 'Discoveries in Photosynthesis', Springer, S. xxvii–xxviii.

Gest, H. & Blankenship, R. E. (2004), 'Time line of discoveries: anoxygenic bacterial photosynthesis', *Photosynthesis Research* **80**, 59–70.

Gest, H. & Kamen, M. D. (1948), 'Studies on the phosphorus metabolism of green algae and purple bacteria in relation to photosynthesis', *Journal of Biological Chemistry* **176**, 299–318.

Glennan, S. (2005), 'Modeling mechanisms', *Studies in History and Philosophy of Biological and Biomedical Sciences* **36**, 443–464.

Goenner, H. (2005), *Einstein in Berlin 1914-1933*, Beck, Munich.

Goenner, H. & Castagnetti, G. (2004), 'Einstein and the Kaiser Wilhelm Institute for Physics: Institutional aims and scientific results', Preprint No. 261, MPI for the History of Science, Berlin.

Govindjee (1995), 'Sixty-three years since Kautsky chlorophyll *a* fluorescence', *Australian Journal of Plant Physiology* **22**, 131–160.

Govindjee (1999), 'On the requirement of minimum number of four versus eight quanta of light for the evolution of one molecule of oxygen in photosynthesis: A historical note', *Photosynthesis Research* **59**, 249–254.

Govindjee (2001), Lighting the Path: A Tribute to Robert Emerson (1903-1959), *in* 'PS2001: Proceedings, 12th International Congress on Photosynthesis, Brisbane, CSIRO', S. (6 page paper). Retrieved September 2008 from the World Wide Web: http://www.publish.csiro.au/ps2001.

Govindjee (2004*a*), Chlorophyll *a* fluorescence: A bit of basics and history, *in* G. C. Papageorgiou & Govindjee, Ed., 'Chlorophyll *a* Fluorescence: A Signature of Photosynthesis', Springer, S. 1–42.

Govindjee (2004*b*), Robert Emerson and Eugene Rabinowitch: Understanding photosynthesis, *in* L. Hoddeson, Ed., 'No Boundaries: University of Illinois Vignettes', University of Illinois Press, Urbana/ Chicago, S. 181–194.

Govindjee (2006), 'Celebrating 20 years of historical papers in Photosynthesis Research', *Photosynthesis Research* **87**, 151–158.

Govindjee (2010), 'Sam Aronoff', *ASPB Newsletter* **37**, 21–22.

Govindjee & Fork, D. (2006), 'Charles Stacy French (1907-1995)', *Biographical Memoirs of the National Academy of Sciences* **88**, 1–29.

Govindjee & Govindjee, R. (1975), Introduction to Photosynthesis, *in* Govindjee, Ed., 'Bioenergetics of Photosynthesis', Academic Press, New York / San Francisco / London, S. 1–50.

Govindjee & Krogmann, D. (2004), 'Discoveries in oxygenic photosynthesis (1727-2003): A perspective', *Photosynthesis Research* **80**, 15–27.

Govindjee & Rabinowitch, E. I. (1960), 'Two forms of chlorophyll *a* in vivo with distinct photochemical functions', *Science* **132**, 355–356.

Govindjee, Allen, J. F. & Beatty, J. T. (2004), 'Celebrating the millennium – historical highlights of photosynthesis research; Part 3', *Photosynthesis Research* **80**, 1–13.

Govindjee, Amesz, J. & Knox, R. S. (1996*a*), 'Photosynthetic Unit: Antenna and Reaction Centers (Special issue dedicated to William Arnold)', Photosynthesis Research 48 (1-2).

Govindjee, Beatty, J. T., Gest, H. & Allen, J. F., Ed. (2005), *Discoveries in Photosynthesis*, Springer, Dordrecht.

Govindjee, Ichimura, S., Cederstrand, C. & Rabinowitch, E. I. (1960*a*), 'Effect of combining far-red light with shorter wave light in the excitation of fluorescence in *Chlorella*', *Archives of Biochemistry and Biophysics* **89**, 322–323.

Govindjee, Knox, R. S. & Amesz, J. (1996*b*), 'Editorial (dedicated to William Arnold)', *Photosynthesis Research* **48**, 1–2.

Govindjee, Owens, O. & Hoch, G. (1963), 'A mass spectroscopic study of the Emerson Enhancement Effect', *Biochim. Biophys. Acta* **75**, 281–284.

Govindjee, R., Govindjee & Hoch, G. (1962), 'The Emerson Enhancement Effect in TPN-photoreduction by spinach chloroplasts', *Biochem. Biophys. Res. Commun.* **9**, 222–225.

Govindjee, R., Govindjee & Hoch, G. (1964), 'Emerson Enhancement Effect in chloroplast reactions', *Plant Physiology* **39**, 10–14.

Govindjee, R., Rabinowitch, E. I. & Govindjee (1968), 'Maximum quantum yield and action spectra of photosynthesis and fluorescence in Chlorella', *Biochim. Biophys. Acta* **162**, 530–544.

Govindjee, R., Thomas, J. B. & Rabinowitch, E. I. (1960*b*), '"Second Emerson Effect" in the Hill reaction of Chlorella cells with quinone as oxidant', *Science* **132**, 421–421.

Grasshoff, G. & Nickelsen, K., Ed. (2001), *Dokumente zur Entdeckung des Harnstoffzyklus. I: Laborbuch Hans Krebs und Erstpublikationen*, Bern Studies in the History and Philosophy of Science, Bern.

Grasshoff, G. & Nickelsen, K., Ed. (2002), *Dokumente zur Entdeckung des Harnstoffzyklus. II: Laborbuch Kurt Henseleit*, Bern Studies in the History and Philosophy of Science, Bern.

Grasshoff, G. (1994), Die Kunst wissenschaftlichen Entdeckens. Grundzüge einer Theorie epistemischer Systeme, Habilitationsschrift, Universität Hamburg.

Grasshoff, G. (1998*a*), Discovery of the Urea Cycle : Computer Models of Scientific Discovery, *in* P. Ahrweiler & N. Gilbert, Ed., 'Computer Simulations in Science and Technology Studies', Springer, Berlin.

Grasshoff, G. (1998*b*), 'Modelling the astrophysical object SS433: Methodology of model construction by a research collective', *Philosophia Naturalis* **35**, 161–199.

Grasshoff, G. (1999), Normal Star Observation in Late Astronomical Babylonian Diaries, *in* N. M. Swerdlow, Ed., 'Ancient Astronomy and Celestial Divination', Dibner Institute Studies in the History of Science and Technology, MIT Press, Cambridge, Mass. / London, S. 97–147.

Grasshoff, G. & May, M. (2001), Causal Regularities, *in* W. Spohn, M. Ledwig & M. Esfeld, Ed., 'Current Issues in Causation', Mentis, Paderborn, S. 85–114.

Grasshoff, G. & May, M. (2003), Hans Krebs and Kurt Henseleit's Laboratory Notebooks and their Discovery of the Urea Cycle: Reconstructed with Computer Models, *in* F. L. Holmes, J. Renn & H.-J. Rheinberger, Ed., 'Reworking the Bench: Research Notebooks in the History of Science', Kluwer Academic Publishers, Dordrecht, S. 269–294.

Grasshoff, G., Casties, R. & Nickelsen, K. (2000), *Zur Theorie des Experiments. Untersuchungen am Beispiel der Entdeckung des Harnstoffzyklus*, Educational Materials, Bern Studies for the History and Philosophy of Science, Bern.

Groeben, C. (1975), *The Naples Zoological Station at the time of Anton Dohrn: Exhibition and Catalogue. In collab. with Irmgard Müller*, Stazione Zoologica, Napoli.

Groeben, C. (2005), Catalysing Science: The *Stazione Zoologica di Napoli* as a Place for the Circulation of Scientific Ideas, *in* C. Groeben, J. Kaasch & M. Kaasch, Ed., 'Places of Biological Research', Verlag für Wissenschaft und Bildung, Berlin, S. 53–64.

Guttman, L., Hess, D. C., Myers, F. E. & Wolfe, H. C. (1970), 'Foster Frederick Rieke 1905-1970', *Journal of Applied Physics* **41**, 3911.

Haber, F. & Willstätter, R. (1931), 'Unpaarigkeit und Radikalketten im Reaktionsmechanismus organischer und enzymatischer Vorgänge', *Berichte der Deutschen Chemischen Gesellschaft* **64**, 2844–2856.

Haberlandt, G. (1881), 'Vergleichende Anatomie des assimilatorischen Gewebesystems der Pflanzen', *Jahrbuch für wissenschaftliche Botanik* **13**, 74–188.

Haberlandt, G. (1884), *Physiologische Pflanzenanatomie*, Engelmann, Leipzig.

Hall, V. M. D. (1980), 'The role of force or power in Liebig's physiological thought', *Medical History* **24**, 20–59.

Hatch, M. D. (2002), 'C_4 photosynthesis: discovery and resolution', *Photosynthesis Research* **73**, 251–256.

Hatch, M. D. & Slack, C. R. (1966), 'Photosynthesis in sugar cane leaves: a new carboxylation reaction and the pathway of sugar formation', *Biochemical Journal* **101**, 103–111.

Hatch, M. D., Slack, C. R. & Johnson, H. S. (1967), 'Further studies on a new pathway of photosynthetic CO_2 fixation in sugarcane and its occurrence in other plant species', *Biochemical Journal* **102**, 417–422.

Haxo, F. T. & Blinks, L. R. (1950), 'Photosynthetic action spectra of marine algae', *Journal of General Physiology* **33**, 389–421.

Heilbron, J. L. & Seidel, R. W. (1989), *Lawrence and his Laboratory: A History of the Lawrence Berkeley Laboratory*, University of California Press, Berkeley.

Heilbron, J. L., Seidel, R. W. & Wheaton, B. R. (1981), *Lawrence and his Laboratory: Nuclear Science at Berkeley*, University of California Press.

Hendricks, S. B. (1953), 'A discussion of photosynthesis', *Science* **117**, 370–373.

Henning, E. (1987), Otto Heinrich Warburg – Der "Kaiser von Dahlem", *in* W. Treue & G. Hildebrandt, Ed., 'Berlinische Lebensbilder. Bd. 1: Naturwissenschaftler', Colloquium Verlag, Berlin, S. 299–316.

Hentschel, K. (2005), *Die Mentalität deutscher Physiker in der frühen Nachkriegszeit (1945-1949)*, Synchron, Heidelberg.

Hentschel, K., Ed. (1996), *Physics and National Socialism. An Anthology of Primary Sources*, Birkhäuser, Basel.

Herken, G. (2002), *Brotherhood of the Bomb. The Tangled Lives and Loyalties of Robert Oppenheimer, Ernest Lawrence, and Edward Teller*, Henry Holt, New York.

Hesse, M. B. (1963), *Models and Analogies in Science*, Sheed and Ward, London.

Hill, R. (1937), 'Oxygen evolved by isolated chloroplasts', *Nature* **139**, 881–882.

Hill, R. (1939), 'Oxygen produced by isolated chloroplasts', *Proceedings of the Royal Society of London, Series B* **127**(847), 192–210.

Hill, R. (1954), 'The cytochrome *b* component of chloroplasts', *Nature* **174**, 501.

Hill, R. (1965), The Biochemists' Green Mansions: The Photosynthetic Electron-Transport Chain in Plants, *in* P. N. Campbell & G. D. Greville, Ed., 'Essays in Biochemistry', Bd. 1, Academic Press, London / New York, S. 121–151.

Hill, R. & Bendall, F. (1960), 'Function of the two cytochrome components in chloroplasts: a working hypothesis', *Nature* **186**, 136–137.

Hill, R. & Bhagvat, K. (1939), 'Cytochrome oxidase in flowering plants', *Nature* **143**, 726.

Hill, R. & Bonner, W. D. (1961), The Nature and Possible Function of Chloroplast Cytochromes, *in* W. D. McElroy & B. Glass, Ed., 'Light and Life', Johns Hopkins Press, Baltimore, S. 424–435.

Hill, R. & Hartree, E. F. (1953), 'Hematin compounds in plants', *Annual Review of Plant Physiology* **4**, 115–150.

Hill, R. & Scarisbrick, R. (1940*a*), 'Production of oxygen by iulluminated chloroplasts', *Nature* **146**, 61–62.

Hill, R. & Scarisbrick, R. (1940*b*), 'The reduction of ferric oxalate by isolated chloroplasts', *Proceedings of the Royal Society of London, Series B* **129**(855), 233–255.

Hill, R. & Scarisbrick, R. (1951), 'The haematin compounds of leaves', *New Phytologist* **50**, 98–111.

Hofmann, K. A. & Schumpelt, K. (1916), 'Bildung von Formaldehyd und anderen organischen Stoffen aus Ameisensäure. I. Mitteilung: Selbstreduktion der Formiate', *Berichte der Deutschen Chemischen Gesellschaft zu Berlin* **49**, 303–317.

Holmes, F. L. (1991), *Hans Krebs: The Formation of a Scientific Life*, Oxford University Press.

Holmes, F. L. (1993), *Hans Krebs: Architect of Intermediary Metabolism, 1933-1937*, Oxford University Press.

Homann, P. H. (2002), 'Hydrogen metabolism of green algae: discovery and early research – a tribute to Hans Gaffron and his coworkers', *Photosynthesis Research* **76**, 93–103.

Hopkins, F. G. (1949), The Dynamic Side of Biochemistry, *in* J. Needham, W. Dunn & E. Baldwin, Ed., 'Hopkins and Biochemistry: 1861-1947', Hefner and Sons, Cambridge, S. 136–159.

Hoppe, B. (1997), Structural Development of Experimental Methods in the Researches on Gas-Exchange and Photosynthesis, *in* B. Hoppe, Ed., 'Biology Integrating Scientific Fundamentals: Contributions to the History of Interrelations between Biology, Chemistry, and Physics from the 18th to the 20th Centuries', Bd. 21 von *Algorismus: Studien zur Geschichte der Mathematik und der Naturwissenschaften*, Institut für Geschichte der Naturwissenschaften, München, S. 10–30.

Horecker, B. L. & Smyrniotis, P. Z. (1952), 'The enzymatic formation of sedoheptulose phosphate from pentose phosophate', *Journal of the American Chemical Society* **74**, 2123.

Horecker, B. L. & Smyrniotis, P. Z. (1953), 'The coenzyme function of thiamine pyrophosphate in pentose phosophate metabolism', *Journal of the American Chemical Society* **75**, 1009–1010.

Horecker, B. L., Hurwitz, J. & Weissbach, A. (1954), 'Pentose phosphate and carbon dioxide fixation with spinach extracts', *Journal of the American Chemical Society* **76**, 3611–3612.

Hungate, R. E. (1986), 'Obituary: Cornelis B. van Niel (1897-1985)', *Photosynthesis Research* **10**, 139–142.

Huzisige, H. & Ke, B. (1993), 'Dynamics of the history of photosynthesis research', *Photosynthesis Research* **38**, 185–209.

Höxtermann, E. (1992), 'Fundamental discoveries in the history of photosynthesis research', *Photosynthetica* **26**, 485–502.

Höxtermann, E. (2001), Otto Heinrich Warburg (1883-1970), *in* I. Jahn & M. Schmitt, Ed., 'Darwin & Co. Eine Geschichte der Biologie in Portraits. Vol. 2', Beck, München, S. 251–274.

Höxtermann, E. (2007), 'A comment on Warburg's early understanding of biocatalysis', *Photosynthesis research* **92**, 121–127.

Höxtermann, E. & Sucker, U. (1989), *Otto Warburg*, Biographien hervorragender Naturwissenschaftler, Techniker und Mediziner; 91, Teubner, Leipzig.

Jaenicke, L. (2007), 'Horst Tobias Witt (1922-2007). Ahnherr der biophysikalischen Photosyntheseforschung in Deutschland', *BIOspektrum* **7**, 815–816.

Jagendorf, A. T. (1956), 'Oxidation and reduction of pyridine nucleotides by purified chloroplasts', *Archives of Biochemistry and Biophysics* **62**, 141–150.

Jagendorf, A. T. (1998), 'Chance, luck and photosynthesis research: An inside story', *Photosynthesis Research* **57**, 215–229.

Jencks, W. P. & Wolfenden, R. F. (2000), 'Fritz Albert Lipmann (1899-1986)', *Biographical Memoirs of Fellows of the Royal Society* **46**, 335–344.

Johnston, H. (2003), *A Bridge Not Attacked. Chemical Warfare Civilian Research During World War II*, World Scientific Publishing, River Edge (New Jersey).

Junge, W. (2005), Protons, proteins and ATP, *in* Govindjee, J. T. Beatty, H. Gest & J. F. Allen, Ed., 'Discoveries in Photosynthesis', Springer, S. 571–595.

Junge, W. & Rutherford, A. W. (2007), 'Obituary: Horst Tobias Witt (1922-2007)', *Nature* **448**, 425.

Kalckar, H. (1937), 'Phosphorylation in kidney tissues', *Enzymologia* **2**, 47–52.

Kalckar, H. (1974), 'Origins of the concept oxidative phosphorylation', *Molecular and Cellular Biochemistry* **5**, 55–62.

Kalckar, H. (1991), '50 years of biological research: From oxidative phosphorylation to energy requiring transport regulation', *Annual Review of Biochemistry* **60**, 1–37.

Kalckar, H. M. (1941), 'The nature of energetic coupling in biological systems', *Chemical Reviews* **28**, 71–178.

Kamen, M. D. (1949), Some Remarks on Tracer Researches in Photosynthesis, *in* J. Franck & W. E. Loomis, Ed., 'Photosynthesis in Plants', State College Press, Ames, Iowa, S. 365–380.

Kamen, M. D. (1974), 'Early days in CO_2 fixation: Some brief comments on the Berkeley experience', *Molecular and Cellular Biochemistry* **5**, 99–101.

Kamen, M. D. (1985), *Radiant Science, Dark Politics. A Memoir of the Nuclear Age*, University of California Press, Berkeley.

Kamen, M. D. (1989), 'Onward in a fabulous half century', *Photosynthesis Research* **21**, 139–144.

Kamp, A. F., Rivière, J. W. M. L. & Verhoeven, W., Ed. (1959), *Albert Jan Kluyver: His Life and Work. Biographical Memoranda, Selected Papers, Bibliography and Addenda*, North-Holland Publishing Company, Amsterdam.

Katz, E. (1949), Chlorophyll Fluorescence as an Energy Flow Meter for Photosynthesis, *in* J. Franck & W. E. Loomis, Ed., 'Photosynthesis in Plants', State College Press, Ames, Iowa, S. 287–292.

Kautsky, H. & Franck, U. (1943), 'Chlorofluoreszenz und Kohlensäureassimilation XI. Die Chlorophyllfluoreszenz von *Ulva lactuca* und ihre Abhängigkeit von Narcotica, Sauerstoff und Kohlenoxyd', *Biochemische Zeitschrift* **315**, 176–206.

Kautsky, H. & Hirsch, A. (1931), 'Neue Versuche zur Kohlensäureassimilation', *Naturwissenschaften* **19**, 964–964.

Kautsky, H., Appel, W. & Amann, H. (1960), 'Chlorophyll-Fluoreszenz und Kohlensäureassimilation', *Biochemische Zeitschrift* **332**, 277–292.

Kautsky, H., de Bruijn, H., Neuwirth, R. & Baumeister, W. (1933), 'Energie-Umwandlungen an Grenzflächen. VII. Mitteilung: Photo-sensibilisierte Oxydation als Wirkung eines aktiven, metastabilen Zustandes des Sauerstoff-Moleküls', *Berichte der Deutschen Chemischen Gesellschaft* **66**, 1588–1600.

Kautsky, H., Hirsch, A. & Davidshöfer, F. (1932), 'Energie-Umwandlungen an Grenzflächen. VI. Mitteilung: Kohlensäure-Assimilation (1.)', *Berichte der Deutschen Chemischen Gesellschaft* **65**, 1762–1770.

Kautsky, H., Hirsch, A. & Davidshöfer, F. (1935), '?', *Biochemische Zeitschrift* **277**, 250.

Kay, L. E. (1993), *The Molecular Vision of Life: Caltech, the Rockefeller Foundation and the Rise of the New Biology*, Oxford University Press, New York.

Keilin, D. (1925*a*), 'A comparative study of turacin and haematin and its bearing on cytochrome', *Proceedings of the Royal Society of London, Series B* **100**, 129–151.

Keilin, D. (1925*b*), 'On cytochrome, a respiratory pigment, common to animals, yeast, and higher plants', *Proceedings of the Royal Society of London, Series B* **98**, 312–339.

Keilin, D. (1966), *The History of Cell Respiration and Cytochrome*, Cambridge UP, Cambridge.

Keilin, D. & Hartree, E. F. (1939), 'Cytochrome and cytochrome oxidase', *Proceedings of the Royal Society of London, Series B* **127**, 167–191.

Keilin, D. & Hill, R. (1933), 'Estimation of haematin iron and the oxidation-reduction equivalent of cytochrome *c*', *Proceedings of the Royal Society of London, Series B* **114**, 104–109.

Keilin, D., Dixon, M. & Hill, R. (1931), 'The absorption spectrum of the component *c* of cytochrome', *Proceedings of the Royal Society of London, Series B* **109**, 29–34.

Kennedy, E. P. (1996), 'Herman Moritz Kalckar (1908-1991)', *Biographical Memoirs of the National Academy of Sciences*.

Kennedy, E. P. (2001), 'Hitler's gift and the era of biosynthesis', *The Journal of Biological Chemistry* **276**, 42619–42631.

Kleinkauf, H., von Döhren, H. & Jaenicke, L. (1988), *The Roots of Modern Biochemistry: Fritz Lipmann's Squiggle and Its Consequences*, Walter de Gruyter, Berlin.

Klein, M. J., Kox, A. J. & Schulmann, R. E. (1993), *The Swiss Years: Correspondence, 1902-1914*, Bd. 5 von *The Collected Papers of Albert Einstein*, Princeton University Press, Princeton.

Klein, M. J., Kox, A. J., Renn, J. & Schulmann, R. (1995), *The Swiss Years: Writings, 1912-1914*, Bd. 4 von *The Collected Papers of Albert Einstein*, Princeton University Press, Princeton.

Klingenberg, M., Müller, A., Schmidt-Mende, P. & Witt, H. T. (1962), 'Changes in absorption during photosynthesis in the ultra-violet spectrum', *Nature* **194**, 379–380.

Kluyver, A. J. & Donker, H. J. L. (1926), 'Die Einheit in der Biochemie', *Chemie der Zelle und Gewebe: Zeitschrift für die Probleme der Gärung, Atmung und Vitaminforschung* **13**, 134–139.

Knox, R. S. (1996), 'Electronic excitation transfer in the photosynthetic unit: Reflections on work of William Arnold', *Photosynthesis Research* **48**, 35–39.

Kohler, R. E. (1973*a*), 'The background to Otto Warburg's conception of the Atmungsferment', *Journal of the History of Biology* **6**(2), 171–192.

Kohler, R. E. (1973*b*), 'The enzyme theory of life and the origins of biochemistry', *Isis* **64**, 171–192.

Kohler, R. E. (1977), 'Rudolf Schoenheimer, isotopic tracers, and the dynamic state', *Historical Studies in the Physical Sciences* **8**, 257–298.

Kohler, R. E. (1982), *From Medical Chemistry to Biochemistry: the Making of a Biomedical Discipline*, Cambridge University Press.

Kohler, R. E. (1991), *Partners in Science. Foundations and Natural Scientists 1900-1945*, University of Chicago Press, Chicago.

Kok, B. (1956), 'On the reversible absorption change at 705nm in photosynthetic organisms', *Biochim. Biophys. Acta* **22**, 399–401.

Kok, B. (1957), 'Light induced absorption changes in photosynthetic organisms', *Acta Botan. Neerl.* **6**, 316–336.

Kok, B. (1959), 'Light induced absorption changes in photosynthetic organisms II. A split beam difference spectrophotometer', *Plant Physiology* **34**, 184–192.

Kok, B. (1960), Efficiency of Photosynthesis, *in* A. Pirson, Ed., 'Handbuch der Pflanzenphysiologie', Springer, Berlin/ Göttingen/ Heidelberg, S. 566–633.

Kok, B. (1961*a*), Does Photosynthesis Require the Interaction of Two Photochemical Steps?, *in* 'Recent Advances in Botany, Volume II: From Lectures and Symposia presented to the IXth International Botanical Congress, Montreal, August, 1959', University of Toronto Press, Toronto, Canada, S. 1071–1072.

Kok, B. (1961*b*), 'Partial purification and determination of oxidation reduction potential of the photosynthetic chlorophyll complex absorbing at 700 mμ', *Biochim. Biophys. Acta* **48**, 527–532.

Kok, B. & Hoch, G. (1961), Spectral Changes in Photosynthesis, *in* W. D. McElroy & B. Glass, Ed., 'Light and Life', Johns Hopkins Press, Baltimore, S. 397–423.

Kornberg, H. L. (2006), 'John Rodney Quayle (1926-2006)', *Photosynthesis Research* **89**, 59–62.

Kortschak, H. P., Hartt, C. E. & Burr, G. O. (1965), 'Carbon dioxide fixation in sugarcane leaves', *Plant Physiology* **40**, 209–213.

Krebs, H. (1974), 'The discovery of carbon dioxide fixation in mammalian tissues', *Molecular and Cellular Biochemistry* **5**, 79–94.

Krebs, H. (1979), *Otto Warburg: Zellphysiologe, Biochemiker, Mediziner 1883-1970*, Grosse Naturforscher; 41, Wissenschaftliche Verlagsgesellschaft, Stuttgart.

Krebs, H. & Henseleit, K. (1932), 'Untersuchungen über die Harnstoffbildung im Tierkörper', *Hoppe-Seyler's Zeitschrift für physiologische Chemie* **210**, 33–66.

Krebs, H. A. & Johnson, W. A. (1937), 'Metabolism of ketonic acids in animal tissues', *Biochemical Journal* **31**, 645–660.

Kresge, N., Simoni, R. D. & Hill, R. (2005), 'The discovery of ferredoxin and its role in photosynthesis: The work of Anthony San Pietro', *Journal of Biological Chemistry* **280**, e48.

Krogmann, D. W. (2000), 'The golden age of biochemical research in photosynthesis', *Photosynthesis Research* **63**, 109–121.

Kuhn, H. G. (1965), 'James Franck (1882-1964)', *Biographical Memoirs of Fellows of the Royal Society* **11**, 51–74.

Larkum, A. W. D. (2003), 'Contributions of Henrik Lundegårdh', *Photosynthesis Research* **76**, 105–110.

Löb, W. (1906), 'Zur Kenntnis der Assimilation der Kohlensäure', *Landwirtschaftliche Jahrbücher* **35**, 541–578.

Lehmann, J. (1968), 'Kohlenstoff-14 und die Assimilation des Kohlendioxids', *Chemie in unserer Zeit* **2**, 67–73.

Lehninger, A. L. (1951), 'Phosphorylation coupled to the oxidation of dihydrophosphopyridine nucleotide', *Journal of Biological Chemistry* **190**, 345–359.

Lemmerich, J. (2007), *Aufrecht im Sturm der Zeit: Der Physiker James Franck (1882-1964)*, Verlag für Geschichte der Naturwissenschaften und Technik, Diepholz / Stuttgart / Berlin.

Levitt, L. S. (1953), 'Photosynthesis as a photoelectric phenomenon', *Science* **118**, 696–697.

Levitt, L. S. (1954), 'The role of magnesium in photosynthesis', *Science* **120**, 33–35.

Liebig, J. (1843), 'Die Wechselwirthschaft', *Annalen der Chemie und Pharmazie* **46**, 58–97.

Lineweaver, H. & Burk, D. (1934), 'The determination of enzyme dissociation constants', *Journal of the American Chemical Society* **56**, 658–666.

Lipmann, F. (1941), 'Metabolic generation and utilization of phosphate bond energy', *Advances in Enzymology* **1**, 99–162.

Lipmann, F. (1971), *Wanderings of a Biochemist*, Wiley-Interscience, New York.

Lipman, T. O. (1967), 'Vitalism and reductionism in Liebig's physiological thought', *Isis* **58**, 167–185.

Loach, P. (1997), 'A remembrance of Melvin Calvin', *Photosynthesis Research* **54**, 1–3.

Loeb, J. (1905), *Studies in General Physiology*, Chicago.

Lohmann, K. (1929), 'Über die Pyrophosphatfraktion im Muskel', *Naturwissenschaften* **17**, 624–625.

Loomis, W. E. (1960), Historical Introduction, *in* A. Pirson & W. Ruhland, Ed., 'Handbuch der Pflanzenphysiologie', Bd. 5, 1, Springer, Berlin / Göttingen / Heidelberg, S. 85–114.

Losada, M., Trebst, A. V. & Arnon, D. I. (1959), 'Photosynthesis by isolated chloroplasts XI: CO_2 assimilation in a reconstituted chloroplast system', *Journal of Biological Chemistry* **235**, 832–839.

Lundegårdh, H. (1954), 'On the oxidation of cytochrome *f* by light', *Physiologia Plantarum* **7**, 375–382.

Machamer, P., Darden, L. & Craver, C. F. (2000), 'Thinking about Mechanisms', *Philosophy of Science* **67**, 1–25.

Macrakis, K. (1993), *Surviving the Swastika. Scientific Research in Nazi Germany*, Oxford University Press, New York / Oxford.

Magee, J. L., de Witt, T. W., Smith, E. C. & Daniels, F. (1939), 'A photocalorimeter. The quantum efficiency of photosynthesis in algae', *Journal of the American Chemical Society* **61**, 3529.

Manning, W. M. (1938), Photosynthesis, *in* 'Third Report of the Committee on Photochemistry Reprint and Circular Series of the National Research Council', S. 117–156.

Manning, W. M., Stauffer, J. F., Duggar, B. M. & Daniels, F. (1938), 'Quantum efficiency of photosynthesis in *Chlorella*', *Journal of the American Chemical Society* **60**, 266–274.

Mann, T. (1964), 'David Keilin, 1887-1963', *Biographical Memoirs of Fellows of the Royal Society* **10**, 183–205.

Martin, A. J. P. & Synge, R. L. M. (1941), 'A new form of chromatogram employing two liquid phases', *Biochemical Journal* **35**, 1358–1368.

Maruyama, K. (1991), 'The discovery of adenosine triphosphate and the establishment of its structure', *Journal of the History of Biology* **24**, 145–154.

Max Delbrück: Oral History Interview with Carolyn Harding, July 14-September 11, 1978, CIT, p. 55, <http://resolver.caltech.edu/CaltechOH:OH_Delbruck_M> (n.d.).

Mayaudon, J. (1957), 'Study of association between the main nucleoprotein of green leaves and carboxydismutase', *Enzymologia* **18**, 345–354.

Mayaudon, J., Benson, A. A. & Calvin, M. (1957), 'Ribulose-1,5-diphosphate from and CO_2 fixation by *Tetragonia expansa* leaves extract', *Biochim. Biophys. Acta* **23**, 342–351.

May, M. (1999), Kausales Schließen. Eine Untersuchung zur kausalen Erklärung und Theorienbildung, Dissertation, University of Hamburg.

McElroy, W. D. & Glass, B., Ed. (1961), *Light and Life*, Johns Hopkins Press, Baltimore.

Meldola, R. (1906), 'The living organism as a chemical agency', *Journal of the Chemical Society. Transactions* **89**, 749–770.

Melis, A. & Buchanan, R. B. (1995), 'A Tribute to Daniel I. Arnon', Photosynthesis Research 46 (1-2). (Special issues dedicated to Daniel Arnon).

Morrison, M. (2006), 'Where have all the theories gone?', *Philosophy of Science* **74**, 195–228.

Mortenson, L. E., Valentine, R. C. & Carnahan, J. E. (1962), 'An electron transport factor from *Clostridium pasteurianum*', *Biochem. Biophys. Res. Commun.* **7**, 448–452.

Morton, O. (2007), *Eating the Sun. How Plants Power the Planet*, Fourth Estate.

Moses, V. & Moses, S. (2000), 'The Calvin Lab: Bio-Organic Chemistry Group at the University of California, Berkeley, 1945-1963. An oral history conducted 1995-1997', Regional Oral History Office, The Bancroft Library, University of California, Berkeley.

Myers, J. (1961), Evidences for a Specific Participation of Chlorophyll *b* in Photosynthesis, *in* 'Recent Advances in Botany, Volume II: From Lectures and Symposia Presented to the IXth International Botanical Congress, Montreal, August, 1959', University of Toronto Press, Toronto, Canada, S. 1073–1076.

Myers, J. (1971), 'Enhancement studies in photosynthesis', *Plant Physiology* **22**, 289–312.

Myers, J. (1974), 'Conceptual developments in photosynthesis', *Plant Physiology* **54**, 420–426.

Myers, J. (1987), 'Bessel Kok (1918-1979)', *Biographical Memoirs of the National Academy of Sciences* **57**, 124–149.

Myers, J. (1994), 'The 1932 experiments', *Photosynthesis Research* **40**, 303–310.

Myers, J. (2002), 'In one era and out the other', *Photosynthesis Research* **73**, 21–28.

Myers, J. & French, C. S. (1960*a*), 'Evidences from action spectra for a specific participation of chlorophyll *b* in photosynthesis', *Journal of General Physiology* **43**, 723–736.

Myers, J. & French, C. S. (1960*b*), 'Relationships between time course, chromatic transient, and enhancement phenomena of photosynthesis', *Plant Physiology* **35**, 963–969.

Nachmansohn, D. (1972), 'Biochemistry as part of my life', *Annual Review of Biochemistry* **41**, 1–28.

Needham, J., Dunn, W. & Baldwin, E., Ed. (1949), *Hopkins and Biochemistry: 1861-1947*, Hefner and Sons, Cambridge.

Nickelsen, K. (2000), *Wissenschaftliche Pflanzenzeichnungen – Spiegelbilder der Natur? Botanische Abbildungen aus dem 18. und frühen 19. Jahrhundert*, Bern Studies in the History and Philosophy of Science, Bern.

Nickelsen, K. (2006*a*), 'Botanists, Draughtsmen and Nature. The Construction of Eighteenth-Century Botanical Illustration', *Studies in History and Philosophy of Biological and Biomedical Sciences* **37**, 1–25.

Nickelsen, K. (2006*b*), *Draughtsmen, Botanists and Nature. The Construction of Eighteenth-Century Botanical Illustrations*, Archimedes, Kluwer Academic, Dordrecht.

Nickelsen, K. (2007), 'Otto Warburg's first approach to photosynthesis', *Photosynthesis Research* **92**, 109–120.

Nickelsen, K. (2008*a*), 'Ein bisher unbekanntes Zeitzeugnis – Otto Warburgs Tagebuchnotizen Feb.-April 1945', *NTM* **16**, 103–115.

Nickelsen, K. (2008*b*), 'From leaves to molecules: Botany and the development of photosynthesis research', *Annals for the History and Philosophy of Biology* **12**, 1–40.

Nickelsen, K. (2009), 'The construction of a scientific model: Otto Warburg and the Building Block Strategy', *Studies in History and Philosophy of Biological and Biomedical Sciences* **40**, 73–86.

Nickelsen, K. & Grasshoff, G. (2008), Concepts from the Bench: Krebs and the Urea Cycle, *in* G. Hon, J. Schickore & F. Steinle, Ed., 'Going Amiss in Experimental Research', Springer, S. 91–117.

Nickelsen, K. & Grasshoff, G. (submitted), 'In pursuit of formaldehyde: Causally explanatory models and falsification', *Studies in History and Philosophy of the Biological and Biomedical Sciences*. (to be submitted).

Nishimura, M. S., Whittingham, C. P. & Emerson, R. (1951), The Maximum Efficiency of Photosynthesis, *in* 'Carbon Dioxide Fixation and Photosynthesis', Cambridge University Press, S. 176–210.

Ochoa, S. (1950), 'Chemical transformations in photosynthesis', *Federation Proceedings* **9**, 551–553.

Ochoa, S. & Vishniac, W. (1952), 'Carboxylation reactions and photosynthesis', *Science* **115**, 297–301.

Ochoa, S., Veiga Salles, J. B. & Ortiz, P. J. (1950), 'Biosynthesis of dicarboxylic acids by carbon dioxide fixation III Enzymatic synthesis of igr-malic acid by reductive carboxylation of pyruvic acid', *Journal of Biological Chemistry* **187**, 863–874.

Ohmura, T. (1955), 'Oxidative and photosynthetic phosphorylation', *Nature* **176**, 467–468.

Oppenheimer, J. R. (1941), 'Internal conversion in photosynthesis (Abstract)', *Physical Reviews* **60**, 158.

Osterhout, W. J. V. (1918), 'A method for studying respiration', *Journal of General Physiology* **1**, 17–22.

Osterhout, W. J. V. (1919), 'Comparative studies on respiration. VII. Respiration and antagonism', *Journal of General Physiology* **2**, 1–3.

Osterhout, W. J. V. & Haas, A. R. C. (1918), 'On the dynamics of photosynthesis', *Journal of General Physiology* **1**, 1–16.

Osterhout, W. J. V. & Haas, A. R. C. (1919), 'The temperature coefficient of photosynthesis', *Journal of General Physiology* **2**, 299–304.

Pauli, W. (1993), *Wissenschaftlicher Briefwechsel mit Bohr, Einstein, Heisenberg u.a.. Vol. 3: 1940-1949*, Springer, Berlin.

Pauly, P. J. (1987), General Physiology and the Discipline of Physiology, 1890-1935, *in* G. L. Geison, Ed., 'Physiology in the American Context, 1850-1940', American Physiological Society, Bethesda, MD, S. 195–208.

Pearlstein, R. M. (1966), Migration and Trapping of Excitation Quanta in Photosynthetic Units, Ph.d., University of Maryland, College Park, Maryland.

Pearlstein, R. M. (1967), 'Migration and trapping of excitation quanta in photosynthetic units', *Brookhaven Symposia in Biology* **19**, 8–15.

Pearlstein, R. M. (2002), 'Photosynthetic exciton theory in the 1960s', *Photosynthesis Research* **73**, 119–126.

Pfeffer, W. (1897), *Pflanzenphysiologie. Ein Handbuch der Lehre vom Stoffwechsel und Kraftwechsel in der Pflanze*, 2., revised and amended Aufl., Engelmann, Leipzig.

Pietro, A. S. (2008), 'Memories: from protein synthesis to photosynthesis', *Photosynthesis Research* **96**, 185–199.

Pirson, A. (1994), 'Sixty years in algal physiology and photosynthesis', *Photosynthesis Research* **40**, 207–221.

Pirson, A., Krollpfeiffer, I. & Schaefer, G. (1953), 'Leistungsfähigkeit und Fehlerquellen manometrischer Stoffwechselmessungen', *Marburger Sitzungsberichte* **76**, 3–27.

Porter, G. (1976), 'Some reflections on the work of Theodor Förster', *Naturwissenschaften* **63**, 207–211.

Pringsheim, E. G. (1970), 'Ernst Georg Pringsheim: Eine autobiographische Skizze', *Med. Hist. J.* **5**, 125–137.

Quayle, J. R., Fuller, R. C., Benson, A. A. & Calvin, M. (1954), 'Enzymatic carboxylation of ribulose diphosphate', *Journal of the American Chemical Society* **76**, 3610–3612.

Rabinowitch, A. (2005), 'Founder and father', *Bulletin of the Atomic Sciences* **61**, 30–37.

Rabinowitch, E. I. (1945), *Photosynthesis and Related Processes. Vol I: Chemistry of Photosynthesis, Chemosynthesis and Related Processes in Vitro and in Vivo*, Interscience Publishers, New York.

Rabinowitch, E. I. (1956), *Photosynthesis and Related Processes. Vol II, 2: Kinetics of Photosynthesis (continued); Addenda*, Interscience Publishers, New York.

Rabinowitch, E. I. (1961), 'Robert Emerson (1903-1959)', *Biographical Memoirs of the National Academy of Sciences* **25**, 112–131.

Rabinowitch, E. I. (1963), The Mechanism of Photosynthesis, *in* 'Studies on Microalgae and Photosynthetic Bacteria', Bd. 373, University of Tokyo Press, S. 112–121.

Rabinowitch, E. I. & Govindjee (1961), Different Forms of Chlorophyll *a* in vivo and their Photochemical Function, *in* W. D. McElroy & B. Glass, Ed., 'Light and Life', Johns Hopkins Press, Baltimore, S. 378–386.

Racker, E., de la Haba, G. & Leder, I. G. (1953), 'Thiamine pyrophosphate, a coenzyme of transketolase', *Journal of the American Chemical Society* **75**, 1010–1011.

Reinhardt, C. (2006), *Shifting and Rearranging. Physical Methods and the Transformation of Modern Chemistry*, Science History Publications, Sagamore Beach.

Renger, G. (2008), 'Horst Tobias Witt (1922-2007)', *Photosynthesis Research* **96**, 5–8.

Renger, G. & Govindjee (1993), 'How Plants and Cyanobacteria Make Oxygen: 25 Years of Period Four Oscillations (Special issue dedicated to Bessel Kok and Pierre Joliot)', Photosynthesis Research 38 (3-4).

Reynolds, P. E. (2007), 'Ernest Frederick Gale: 15 July 1914 - 7 March 2005', *Biographical Memoirs of Fellows of the Royal Society* **53**, 143–161.

Rheinberger, H.-J. (1997), *Toward a History of Epistemic Things: Synthesizing Proteins in the Test Tube*, Stanford University Press, Stanford.

Rhodes, R. (1986), *The Making of the Atomic Bomb*, Simon and Schuster.

Rich, P. (1992), 'Special issue dedicated to Robin Hill', Photosynthesis Research 34 (3).

Rieke, F. F. (1939), 'On the quantum efficiency of photosynthesis', *Journal of Chemical Physics* **7**, 238–244.

Rieke, F. F. (1949), Quantum Efficiencies for Photosynthesis and Photoreduction in Green Plants, *in* J. Franck & W. E. Loomis, Ed., 'Photosynthesis in Plants', Iowa State College Press, Ames, Iowa, S. 251–272.

Robinson, G. W. (1967), 'Excitation transfer and trapping in photosynthesis', *Brookhaven Symposia in Biology* **19**, 16–48.

Rosenberg, J. L. (2004), 'The contributions of James Franck to photosynthesis research: a tribute', *Photosynthesis Research* **80**, 71–76.

Rürup, R. (2008), *Schicksale und Karrieren. Gedenkbuch für die von den Nationalsozialisten aus der Kaiser-Wilhelm-Gesellschaft vertriebenen Forscherinnen und Forscher*, (in collaboration with Michael Schüring), Wallstein.

Rüskamp, W., Ed. (1989), *Eine vollkommene Närrin durch meine ewigen Gefühle. Aus den Tagebüchern der Lotte Warburg 1925 bis 1947*, Druckhaus Bayreuth.

Ruben, S. (1943), 'Photosynthesis and phosphorylation', *Journal of the American Chemical Society* **65**, 279.

Ruben, S. & Kamen, M. D. (1940*a*), 'Photosynthesis with radioactive carbon IV. Molecular weight of the intermediates and a tentative theory of photosynthesis', *Journal of the American Chemical Society* **62**, 3451–3455.

Ruben, S. & Kamen, M. D. (1940*b*), 'Radioactive carbon in the study of respiration in heterotrophic systems', *Proceedings of the National Academy of Sciences (USA)* **26**, 418–422.

Ruben, S. & Kamen, M. D. (1941), 'Long-lived radioactive carbon: C14', *Physical Review* **59**, 349–354.

Ruben, S., Hassid, W. Z. & Kamen, M. D. (1939*a*), 'Radioactive carbon in the study of photosynthesis', *Journal of the American Chemical Society* **61**, 661–663.

Ruben, S., Kamen, M. D. & Hassid, W. Z. (1940*a*), 'Photosynthesis with radioactive carbon II. Chemical properties of the intermediates', *Journal of the American Chemical Society* **62**, 3443–3450.

Ruben, S., Kamen, M. D. & Perry, L. H. (1940*b*), 'Photosynthesis with radioactive carbon III. Ultracentrifugation of intermediate products', *Journal of the American Chemical Society* **62**, 3450–51.

Ruben, S., Kamen, M. D., Hassid, W. Z. & DeVault, D. C. (1939*b*), 'Photosynthesis with radio-carbon', *Science* **90**, 570–571.

Ruben, S., Randall, M., Kamen, M. D. & Hyde, J. L. (1941), 'Heavy oxygen as a tracer in the study of photosynthesis', *Journal of the American Chemical Society* **63**, 877–879.

Sachs, J. (1862), 'Über den Einfluss des Lichtes auf die Bildung des Amylums in den Chlorophyllkörnern', *Botanische Zeitung* **20**, 365–373.

Sachs, J. (1864), 'Über die Auflösung und Wiederbildung des Amylums in den Chlorophyllkörnern bei wechselnder Beleuchtung', *Botanische Zeitung* **22**, 189–294.

San Pietro, A. & Lang, H. M. (1956), 'Accumulation of reduced pyridine nucleotides by illuminated grana', *Science* **124**, 118–119.

San Pietro, A. & Lang, H. M. (1958), 'Photosynthetic pyridine nucleotide reductase I. Partial purification and properties of the enzyme from spinach', *Journal of Biological Chemistry* **231**, 211–229.

Schoenheimer, R. L. (1940), *The Dynamic State of Body Constituents*, Cambridge University Press, Cambridge.

Schoenheimer, R. L. & Rittenberg, D. (1935), 'Deuterium as an indicator in the study of intermediary metabolism. I.', *Journal of Biological Chemistry* **111**, 163–168.

Schoenheimer, R. L. & Rittenberg, D. (1937), 'Deuterium as an indicator in the study of intermediary metabolism. XI. Further studies on the biological uptake of deuterium into organic substances, with special reference to fat and cholesterol formation', *Journal of Biological Chemistry* **121**, 235–253.

Schroeder, H. (1917), *Die Hypothesen über die chemischen Vorgänge bei der Kohlensäure-Assimilation und ihre Grundlagen*, Fischer, Jena.

Schulmann, R., Kox, A. J., Janssen, M. & Illy, J. E. (1998), *The Berlin Years: Correspondence, 1914-1918*, Bd. 5 von *The Collected Papers of Albert Einstein*, Princeton University Press, Princeton.

Seaborg, G. T. & Benson, A. A. (1997), 'Melvin Calvin (1911-1997)', *Biographical Memoirs of the National Academy of Sciences* **75**, 2–21.

Shen, Y.-K. & Wei, J.-M. (1998), The Discovery of Photophosphorylation, *in* S.-D. Kung & S.-F. Yang, Ed., 'Discoveries in Plant Biology', Bd. 2, World Scientific Publishing, Singapore etc., S. 105–114.

Simoni, R. D., Hill, R. L. & Vaughan, M. (2002), 'The use of isotope tracers to study intermediary metabolism: Rudolf Schoenheimer', *The Journal of Biological Chemistry* **43**, e1–e2 (http://www.jbc.org/cgi/reprint/277/43/e31).

Singleton, R. (1997), 'Heterotrophic CO_2-fixation, mentors, and students: The Wood-Werkman reactions', *Journal of the History of Biology.* **30**, 91–120.

Sloan, P. R. (2009), Biophysics in Berlin, *in* P. R. Sloan & B. Fogel, Ed., 'Creating a Physical Biology: The Three-Man Paper and the Origins of Molecular Biology', University of Chicago Press, Chicago, S. 77–124.

Spath, S. B. (1999), C. B. van Niel and the Culture of Microbiology, 1920-1965, Thesis (ph. d.), University of California, Berkeley.

Stachel, J., Cassidy, D. C., Renn, J. & Schulmann, R., Ed. (1989), *The Collected Papers of Albert Einstein. Vol 2: The Swiss Years: Writings, 1900-1909*, Princeton University Press.

Stemler, A. J. (2002), 'The bicarbonate effect, oxygen evolution, and the shadow of Otto Warburg', *Photosynthesis Research* **73**(73), 177–183.

Stepka, W., Benson, A. A. & Calvin, M. (1948), 'The path of carbon in photosynthesis: II. Amino acids', *Science* **108**, 304.

Stiles, W. (1925), *Photosynthesis: The Assimilation of Carbon by Green Plants*, Longmans/ Green, London.

Stoll, A. (1932), 'Über den chemischen Verlauf der Photosynthese', *Naturwissenschaften* **20**, 955–958.

Strehler, B. L. (1951), 'The luminescence of isolated chloroplasts', *Archives for Biochemistry and Biophysics* **34**, 239–248.

Strehler, B. L. (1952), Photosynthesis – Energetics and Phosphate Metabolism, *in* W. D. McElroy & H. B. Glass, Ed., 'Phosphorus Metabolism II', Johns Hopkins Press, Baltimore, S. 491–502.

Strehler, B. L. (1953), 'Firefly Luminescence in the Study of Energy Transfer Mechanisms II: Adenosine Triphosphate and Photosynthesis', *Archives of Biochemistry and Biophysics* **43**, 67–79.

Strehler, B. L. (1996), 'Halcyon days with Bill Arnold', *Photosynthesis Research* **48**, 11–18.

Strehler, B. L. & Arnold, W. A. (1951), 'Light production by green plants', *Journal of General Physiology* **34**, 809–820.

Strehler, B. L. & Hendley, D. (1961), Continuous measurement of photosynthetic phosphorylation with the firefly luminescence assay system, *in* W. D. McElroy & B. Glass, Ed., 'Light and Life', Johns Hopkins Press, Baltimore, S. 601–608.

Strehler, B. L. & McElroy, W. D. (1949), 'Purification of firefly luciferin', *Journal of Cell Compounds* **34**, 457–466.

Strehler, B. L. & Totter, J. (1951), 'Firefly luminescence in the study of energy transfer mechanisms I. Substrate and enzyme determination', *Archives of Biochemistry and Biophysics* **40**, 28–41.

Sucker, U. (2002), *Das Kaiser-Wilhelm-Institut für Biologie. Seine Gründungsgeschichte, seine problemgeschichtlichen und wissenschaftstheoretischen Voraussetzungen (1911-1916)*, Steiner, Stuttgart.

Tagawa, K. & Arnon, D. I. (1962), 'Ferredoxin as electron carrier in photosynthesis and in the bacterial production and consumption of hydrogen gas', *Nature* **195**, 537–543.

Tanada, T. (1951), 'The photosynthetic efficiency of carotenoid pigments in *Navicula minima*', *American Journal of Botany* **38**, 276–283.

Teich, M. (1981), 'Ferment or enzyme: What's in a name?', *History and Philosophy of the Life Sciences* **2**, 193–215.

Thimann, K. V. (1938), 'The absorption of carbon dioxide in photosynthesis', *Science* **88**, 506–507.

Thorhaug, A. & Berlyn, G. (2009), 'A tribute to Lawrence Rogers Blinks (1900-1989): light and algae', *Photosynthesis Research* **100**, 129–141.

Thunberg, T. (1923), 'Über einen neuen Weg von der Kohlensäure zum Formaldehyd. Ein Beitrag zur Theorie der Kohlensäureassimilation', *Zeitschrift für Physikalische Chemie* **106**, 305–312.

Timofeeff-Ressovsky, N. W., Zimmer, K. G. & Delbrück, M. (1935), 'Über die Natur der Genmutation und der Genstruktur', *Nachrichten der Gesellschaft der Wissenschaften zu Göttingen, math-physikal. Klasse* **1**, 189–245.

TOLMACH, L. J. (1951*a*), 'Effects of triphosphopyridine nucleotide upon oxygen evolution and carbon dioxide fixation by illuminated chloroplasts', *Nature* **167**, 946–948.

TOLMACH, L. J. (1951*b*), 'The influence of triphosphopyridine nucleotide and other physiological substances upon oxygen evolution from illuminated chloroplasts', *Archives of Biochemistry and Biophysics* **33**, 120–142.

TREBST, A. V., LOSADA, M. & ARNON, D. I. (1960), 'Photosynthesis by isolated chloroplasts XII: Inhibitors of CO_2 assimilation in a reconstituted chloroplast system', *Journal of Biological chemistry* **235**, 840–844.

TREBST, A. V., MANUEL, L. & ARNON, D. I. (1959), 'Photosynthesis by isolated chloroplasts X. Dependence of carbon dioxide assimilation on the photochemical reactions of chloroplasts', *Journal of Biological Chemistry* **234**, 3055–3058.

USHER, F. L. & PRIESTLEY, J. H. (1906), 'A study of the mechanism of carbon assimilation in green plants', *Proceedings of the Royal Society of London, Series B* **77**(518), 369–376.

VAN ITERSON, G., DEN DOOREN DE JONG, L. E. & KLUYVER, A. J. (1940), *Martinus Willem Beijerinck, his Life and Work*, Nijhoff, Hague.

VAN NIEL, C. B. (1930), Photosynthesis in Bacteria, *in* 'Contributions to Marine Biology', Bd. 3, Stanford University Press, S. 161–169.

VAN NIEL, C. B. (1931), 'On the morphology and physiology of the purple and green sulphur bacteria', *Archiv für Mikrobiologie* **3**, 1–112.

VAN NIEL, C. B. (1935), Photosynthesis of Bacteria, *in* R. G. HARRIS, Ed., 'Cold Spring Harbor Symposia on Quantitative Biology, Vol. 3', The Biological Laboratory, New York, S. 138–150.

VAN NIEL, C. B. (1941), 'The bacterial photosynthesis and their importance for the general problem of phtosynthesis', *Advances in Enzymology* **1**, 263–328.

VAN NIEL, C. B. (1949), 'The Delft School and the rise of general microbiology', *Bacteriological Reviews* **13**, 161–174.

VAN NIEL, C. B. (1967), 'The education of a microbiologist: some reflections', *Annual Review of Microbiology* **21**, 1–30.

VAN RENSEN, J. J. S., XU, C. & GOVINDJEE (1999), 'Role of bicarbonate in the photosystem II, the water-plastoquinone oxido-reductase of plant photosynthesis', *Physiologia Plantarum* **105**, 585–592.

VERNON, L. P. (1953), 'Cytochrome *c* content of Rhodospirillum rubrum', *Archives of Biochemistry and Biophysics* **43**, 180–194.

VERNON, L. P. & KAMEN, M. D. (1953), 'Photoauto-oxidation of ferrocytochrome *c* by sonic extracts of Rhodospirillum rubrum', *Archives of Biochemistry and Biophysics* **44**, 298–311.

VERNON, L. P. & ZAUGG, W. S. (1960), 'Photoreductions by fresh and aged chloroplasts: requirement for ascorbate and 2, 6-dichlorophenolindophenol with aged chloroplasts', *Journal of Biological Chemistry* **235**, 2728–2733.

VIERHAUS, R. & BROCKE, B. V., Ed. (1990), *Forschung im Spannungsfeld von Politik und Gesellschaft. Geschichte und Struktur der Kaiser-Wilhelm- / Max-Planck-Gesellschaft*, Stuttgart.

VINOGRADOV, A. P. & TEISS, R. V. (1941), 'Isotope composition of oxygen from different sources', *Dokl. Akad. Nauk SSSR* **33**, 497–501.

VINOGRADOV, A. P. & TEISS, R. V. (1947), 'Novel determination of the isotope composition of oxygen in photosynthesis', *Dokl. Akad. Nauk SSSR* **56**, 57–58.

VISHNIAC, W. & OCHOA, S. (1951), 'Photochemical reduction of pyridine nucleotides by spinach grana and coupled carbon dioxide fixation', *Nature* **167**, 768–769.

VISHNIAC, W. & OCHOA, S. (1952*a*), 'Fixation of carbon dioxide coupled to photochemical reduction of pyridine nucleotides by chloroplast preparations', *Journal of Biological Chemistry* **195**, 75–93.

VISHNIAC, W. & OCHOA, S. (1952*b*), 'Phosphorylation coupled to photochemical reaction of pyridine nucleotides by chloroplast preparations', *Journal of Biological Chemistry* **198**, 501–506.

VON GERHARD, F. (2004), 'Hans Kautsky (1891-1966)', *Chemie in unserer Zeit* **15**, 197–200.

VON MOHL, H. (1837), *Untersuchungen über die anatomischen Verhältnisse des Chlorophylls*, Tübingen.

WALKER, D. A. (1992), 'Robert Hill', *Photosynthesis Research* **34**, 337–338.

WALKER, D. A. (1995), 'One thing leading to another', *Photosynthesis Research* **46**, 45–46.

WALKER, D. A. (1997), 'Tell me where all past years are', *Photosynthesis Research* **51**, 1–26.

WALKER, D. A. (2002), '"'And whose bright presence" – an appreciation of Robert Hill and his reaction', *Photosynthesis Research* **73**, 51–54.

WALKER, D. A. (2007), 'From Chlorella to chloroplasts: A personal note', *Photosynthesis Research* **92**, 181–185.

WARBURG, E. (1900), 'Über die Bildung des Ozons bei der Spitzenentladung in Sauerstoff', *Sitzungsberichte der Königlich Preussischen Akademie der Wissenschaften (Berlin). Phys.-math. Klasse* **II**, 712–721.

WARBURG, E. (1909), 'Einige Bemerkungen über photochemische Wirkung II', *Verhandlungen der Deutschen Physikalischen Gesellschaft.*

WARBURG, E. (1912), 'Über den Energieumsatz bei photochemischen Vorgängen in Gasen II', *Sitzungsberichte der Königlich Preussischen Akademie der Wissenschaften (Berlin)* S. 216–225.

WARBURG, E. (1913), 'Über den Energieumsatz bei photochemischen Vorgängen in Gasen III', *Sitzungsberichte der Königlich Preussischen Akademie der Wissenschaften (Berlin)* S. 644–659.

WARBURG, E. (1917), 'Über die Anwendung der Quantenhypothese auf die Photochemie', *Naturwissenschaften* **5**(30), 489–494.

WARBURG, E. (1920*a*), 'Quantentheoretische Grundlagen der Photochemie', *Zeitschrift für Elektrochemie* **26**, 54–59.

WARBURG, E. (1924), 'Die Quantenregeln in der Photochemie', *Naturwissenschaften* **12**(47), 1058–1063.

WARBURG, E., LEITHÄUSER, G. & JOHANSEN, E. (1907), 'Über das Vakuumbolometer', *Annalen der Physik* **24**, 25–42.

WARBURG, O. (1908), 'Beobachtungen über die Oxydationsprozesse im Seeigelei', *Hoppe-Seyler's Zeitschrift für physiologische Chemie* **57**, 1–16.

WARBURG, O. (1914), 'Beiträge zur Physiologie der Zelle, insbesondere über die Oxydationsgeschwindigkeit in Zellen', *Ergebnisse der Physiologie* **14**, 253–337.

WARBURG, O. (1919), 'Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen I', *Biochemische Zeitschrift* **100**, 230–270.

WARBURG, O. (1920*b*), 'Über die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen. II.', *Biochemische Zeitschrift* **103**, 188–217.

WARBURG, O. (1921), 'Theorie der Kohlensäureassimilation', *Naturwissenschaften* **9**, 354–358.

WARBURG, O. (1925), 'Versuche über die Assimilation der Kohlensäure', *Biochemische Zeitschrift* **166**, 386–406.

WARBURG, O. (1926), Methodische Vorbemerkungen, *in* O. WARBURG, Ed., 'Über den Stoffwechsel der Tumoren. Arbeiten aus dem Kaiser-Wilhelm-Institut für Biologie, Berlin-Dahlem', Springer, Berlin, S. 1–11.

WARBURG, O. (1927), Über die katalytischen Wirkungen der lebendigen Substanz, *in* 'Über die katalytischen Wirkungen der lebendigen Substanz. Arbeiten aus dem Kaiser-Wilhelm-Institut für Biologie, Berlin-Dahlem', Springer, Berlin, S. 1–13.

WARBURG, O. (1945), 'Über den Quantenbedarf der Kohlensäureassimilation', *Naturwissenschaften* **33**, 122–122.

WARBURG, O. (1947), Kohlensäureassimilation I. Der Quantenbedarf der Photochemischen Reduktion der Kohlensäure, *in* R. KUHN, Ed., 'Naturforschung und Medizin in Deutschland 1939-1946. Vol. 39: Biochemie', Wiesbaden, S. 201–210.

WARBURG, O. (1948), 'Assimilatory quotient and photochemical yield', *American Journal of Botany* **35**, 194–204.

WARBURG, O. (1951), 'Ein-Quantenmechanismus der Photosynthese', *Angewandte Chemie* **63**, 282–283.

WARBURG, O. (1952), 'Energetik und Photosynthese', *Naturwissenschaften* **39**, 337–341.

WARBURG, O. (1958), 'Photosynthesis. Experiments at the Max Planck Institute for Cell Physiology, Berlin-Dahlem', *Science* **128**(3315), 68–73.

WARBURG, O. & BURK, D. (1950*a*), '1-Quanten-Mechanismus und Energie-Kreisprozess bei der Photosynthese', *Naturwissenschaften* **37**, 560.

WARBURG, O. & BURK, D. (1950*b*), 'The maximum efficiency of photosynthesis', *Archives of Biochemistry* **25**, 410–443.

WARBURG, O. & CHRISTIAN, W. (1939), 'Isolierung und Kristallisation des Proteins des oxydierenden Gärungsferments', *Biochemische Zeitschrift* **303**, 40–68.

WARBURG, O. & KRIPPAHL, G. (1958), 'Hill-Reaktionen', *Zeitschrift für Naturforschung B* **13**, 509–514.

WARBURG, O. & LÜTTGENS, W. (1944*a*), 'Experiment zur Assimilation der Kohlensäure', *Naturwissenschaften* **32**, 161–161.

WARBURG, O. & LÜTTGENS, W. (1944*b*), 'Weitere Experimente zur Kohlensäureassimilation', *Naturwissenschaften* **32**, 301–301.

WARBURG, O. & NEGELEIN, E. (1922), 'Über den Energieumsatz bei der Kohlensäureassimilation', *Zeitschrift für Physikalische Chemie* **102**, 235–266.

Warburg, O. & Negelein, E. (1923), 'Über den Einfluss der Wellenlänge auf den Energieumsatz bei der Kohlensäureassimilation', *Zeitschrift für Physikalische Chemie* **106**, 191–218.

Warburg, O. & Uyesugi, T. (1924), 'Über die Blackmansche Reaktion', *Biochemische Zeitschrift* **146**, 486–492.

Warburg, O., Burk, D., Schocken, V. & Hendricks, S. (1950), 'The quantum efficiency of photosynthesis', *Biochim. Biophys. Acta* **4**, 335–349.

Warburg, O., Geleick, H. & Briese, K. (1952), 'Über die Messung der Photosynthese in Carbonat-Bicarbonat-Gemischen', *Zeitschrift für Naturforschung* **7b**, 141.

Warburg, O., Krippahl, G. & Schröder, W. (1954), 'Katalytische Wirkung des blaugrünen Lichts auf den Energieumsatz bei der Photosynthese', *Zeitschrift für Naturforschung* **9b**, 667–675.

Warburg, O., Krippahl, G. & Schröder, W. (1955), 'Wirkungsspektrum eines Photosynthese-Fermentes', *Zeitschrift für Naturforschung* **10b**, 631–639.

Warburg, O., Krippahl, G. & Schröder, W. (1956), 'Über den chemischen Mechanismus der Kohlesäureassimilation', *Naturwissenschaften* **43**, 237–241.

Wassink, E. C. (1951), 'Chlorophyll fluorescence and photosynthesis', *Advances in Enzymology* **11**, 91–199.

Wassink, E. C. & Kersten, J. A. (1946), 'The absorption spectrum and the role of carotenoids in the photosynthesis of diatoms', *Enzymologia* **12**, 3.

Weber, M. (2008), 'Causes without mechanisms: Experimental regularities, physical laws and neuroscientific explanation', *Philosophy of Science* **75**, 995–1007.

Weigert, F. (1911), *Die chemischen Wirkungen des Lichts*, Enke, Stuttgart.

Weigert, F. (1912), 'Der Ausnutzungsfaktor der Lichtenergie', *Zeitschrift für wissenschaftliche Photographie, Photophysik und Photochemie* **11**, 381–394.

Weissbach, A., Horecker, B. L. & Hurwitz, J. (1956), 'The enzymatic formation of phosphoglyceric acid from ribulose diphosphate and carbon dioxide', *Journal of Biological Chemistry* **218**, 795–810.

Weller, S. & Franck, J. (1941), 'Photosynthesis in flashing light', *Journal of Physical Chemistry* **45**, 1359–1373.

Werner, P. (1988), *Otto Warburg. Von der Zellphysiologe zur Krebsforschung*, Neues Leben, Berlin.

Werner, P. (1991), *Ein Genie irrt seltener. Otto Heinrich Warburg, ein Lebensbild in Dokumenten*, Akademie Verlag, Berlin.

Werner, P. (1996), *Otto Warburgs Beitrag zur Atmungstheorie: Das Problem der Sauerstoffaktivierung*, Basiliskenpresse, Marburg.

Werner, P. (1997), 'Learning from an adversary? Warburg against Wieland', *Historical Studies in the Physical and Biological Sciences* **28**(1), 173–196.

Werner, P. (2001), 'Humboldt als Vermittler: Schleiden und Mohl contra Liebig', *History and Philosophy of the Life Sciences* **23**, 213–257.

Werner, P. & Holmes, F. L. (2002), 'Justus Liebig and the plant physiologists', *Journal of the History of Biology* **35**, 421–441.

Werner, P. & Irmscher, A., Ed. (1995), *Fritz Haber. Briefe an Richard Willstätter 1910-1934*, VWR Engel, Berlin.

Whatley, F. R. (1995), 'Photosynthesis by isolated chloroplasts: The early work at Berkeley', *Photosynthesis Research* **46**, 17–26.

Whatley, F. R., Allen, M. B. & Arnon, D. I. (1957), 'Cofactors of photosynthetic phosphorylation', *Plant Physiology (suppl.)* **32**, iii.

Whatley, F. R., Allen, M. B., Rosenberg, L. L., Capindale, J. B. & Arnon, D. I. (1956), 'Photosynthesis in isolated chloroplasts V. Phosphorylation and carbon dioxide fixation by broken chloroplasts', *Biochim. Biophys. Acta* **20**, 462–468.

Wiesen, J. (2000), The Richard Willstätter Controversy: The Legacy of Anti-Semitism in the West-German Chemical Industry, *in* J. E. Lesch, Ed., 'The German Chemical Industry in the Twentieth Century', Kluwer, Dordrecht etc., S. 347–366.

Wildman, S. G. (1992), 'Early events along the trail leading to identification of Rubisco', *Protein Science* **1**, 303–304.

Wildman, S. G. (2002), 'Along the trail from Fraction I protein to Rubisco', *Photosynthesis Research* **73**, 243–250.

Wildman, S. G. & Bonner, J. (1947), 'The proteins of green leaves. I. Isolation of enzymatic properties and auxin content of spinach cytoplasmic proteins', *Archves of Biochemistry* **14**, 381–413.

Willstätter, R. (1933), 'Zur Erklärung der Photoreduktion von Kohlensäure durch Chlorophyll', *Naturwissenschaften* **21**, 252–253.

Willstätter, R. (1973), *Aus meinem Leben. Von Arbeit, Musse und Freunden*, 2. Aufl., Verlag Chemie, Weinheim / Bergstr.

Willstätter, R. & Stoll, A. (1913), *Untersuchungen über Chlorophyll. Methoden und Ergebnisse*, Springer, Berlin.

Willstätter, R. & Stoll, A. (1918), *Untersuchungen über die Assimilation der Kohlensäure. Sieben Abhandlungen*, Springer, Berlin.

Wilson, A. T. & Calvin, M. (1955), 'The photosynthetic cycle. CO_2 dependent transients', *Journal of the American Chemical Society* **77**, 5948–5957.

Witt, H. T. (1991), 'Functional mechanism of water splitting photosynthesis', *Photosynthesis Research* **29**, 55–77.

Witt, H. T., Müller, A. & Rumberg, B. (1961*a*), 'Experimental evidence for the mechanism of photosynthesis', *Nature* **191**, 194–195.

Witt, H. T., Müller, A. & Rumberg, B. (1961*b*), 'Oxidized cytochrome and chlorophyll in photosynthesis', *Nature* **192**, 967–969.

Wohl, K. (1940), 'The mechanism of photosynthesis in green plants', *New Phytologist* **39**, 33–64.

Wohl, K. (1941), 'On the mechanism of photosynthesis in purple bacteria and green plants', *New Phytologist* **40**, 34–55.

Wood, H. G. & Werkman, C. H. (1935), 'The utilization of CO_2 by the propionic acid bacteria in the dissimilation of glycerol', *Journal of Bacteriology* **30**, 332.

Wood, H. G. & Werkman, C. H. (1936), 'The utilization of CO_2 in the dissimilation of glycerol by the propionic acid bacteria', *Biochemical Journal* **30**, 48–53.

WOOD, H. G. & WERKMAN, C. H. (1938), 'The utilization of CO_2 by the propionic acid bacteria', *Biochemical Journal* **32**, 1262–1271.

WOODS, D. D. (1957), 'Albert Jan Kluyver (1888-1956)', *Biographical Memoirs of Fellows of the Royal Society* **3**, 109–128.

WURMSER, R. (1921), 'Recherches sur l'assimilation chlorophyllienne', *Archives de Physique Biologique* **1**, 33–141.

WURMSER, R. (1926), 'La loi de l'équivalent photochimique dans la photosynthèse chlorophyllienne', *Le Journal de Physique et le Radium* **7**, 33–44.

WURMSER, R. (1930), *Oxydations et Réductions*, Presses Universitaires de France, Paris.

YODH, A. G. & TROMBERG, B. J. (2000), 'Celebrating Britton Chance', *Journal of Biomedical Optics* **5**, 115–118.

ZALLEN, D. T. (1993*a*), 'The "light" organism for the job: Green algae and photosynthesis research', *Journal of the History of Biology* **26**, 269–279.

ZALLEN, D. T. (1993*b*), 'Redrawing the boundaries of molecular biology: The case of photosynthesis', *Journal of the History of Biology* **26**, 65–87.