Chapter 3
Otto Warburg and the Turn to Manometry (1912–1925)

In this chapter the focus shifts from the collective attempts to explain the mechanism of photosynthesis to a close-up of a particular individual, namely the German cell physiologist and biochemist Otto Warburg. His contributions to the field mark a turning point in twentieth-century photosynthesis research: Warburg 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 added a completely new perspective to the subject by attempting to establish the efficiency of the process in terms of the minimum quantum requirement of photosynthesis.

The example of Warburg illustrates the enormous potential of research opportunistic behaviour in the sense that was introduced in the previous chapter. The application of one experimental technique, measuring metabolic processes using manometric methods, dominated virtually Warburg's 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 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 "investigative pathway", a term that was coined by Frederic L. Holmes[1], that brought Warburg to make his contributions to the field of photosynthesis research, including the question which sources he used to compose his model hypothesis and why Warburg chose 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 subgoals 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 2). How Warburg ingeniously availed himself of fragments taken from these

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[1] See Holmes (2004).

K. Nickelsen, *Explaining Photosynthesis,* History, Philosophy and Theory of the Life Sciences 8, DOI 10.1007/978-94-017-9582-1_3

contexts and recombined them in a new and innovative way is an instructive example of the successful use of conceptual and methodical building blocks.[2] I shall begin by outlining Warburg's career up until 1920, focusing on those details that are of most significance for the aim of this chapter.

3.1 New Materials and Methods

3.1.1 Otto Warburg (1883–1970)

Otto Warburg was one of the most successful and influential biochemists of the twentieth century.[3] Born in 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, who had converted to protestantism before Otto was born. In 1905, after having held various academic positions (in Strasbourg, Freiburg (Breisgau) and Berlin), Emil Warburg was appointed as the President of the renowned *Physikalisch-Technische-Reichsanstalt* (PTR) in Berlin, where he remained for the rest of his working life, that is, until 1922. This Berlin-based appointment would have a momentous influence on the career of his first child and the only son.

Otto Warburg had a typical middle-class German education, attending a humanistic *Gymnasium*, before going on to study chemistry at Freiburg (Breisgau) in 1901. After having spent some time there, Warburg moved to Berlin, where he continued his studies in the laboratory of the organic chemist Emil Fischer (the same Fischer who had contributed in establishing the path of carbon to sugars via formaldehyde; see Chapter 2). While working in Fischer's laboratory, Warburg earned his doctoral degree in chemistry in 1906.[4] In the years before, Warburg had 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

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

[3] For general accounts of Otto Warburg's life and work, see Krebs (1979); Henning (1987); Höxtermann and 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 the physiologists Jacques Loeb, Leonor Michaelis and Otto Meyerhof is treated in Werner (1996), while Kohler (1973a) investigates the background of Warburg's concept of *Atmungsferment*. On Warburg's experimental methods in photosynthesis, see also Hoppe (1997a, pp. 19–20).

[4] 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 and 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 (Phil. Fak. No. 411, folio 210).

measure light intensities.[5] This was one of the measuring instruments that Otto Warburg would later use in his research work on the quantum yield of photosynthesis.[6]

Warburg could then have embarked upon a career as a chemist. Instead, 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 doctorate in medicine in 1911, and in 1912 attained his habilitation. Warburg stayed with Krehl for 1 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 in Physiology or Medicine in 1931, largely because of the studies in cell oxidation, which he resumed in the 1920s.

In 1913, at the age of 30, 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 the Dahlem district of the city.[7] 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 kept himself busy 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 Wilhelms University, where Warburg apparently worked on the oxidation potentials of living cells.[8] Both these topics 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. Although Warburg remained in service until 1918, 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.[9] 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

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[5] See, e.g., Warburg et al. (1907) and Warburg (1909). On the PTR's history, see Cahan (1989).

[6] For an autobiographical account of this period, see the taped interview with Warburg of 1966, quoted in Krebs (1979, pp. 94–95).

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

[8] See Werner (1991, pp. 75 and 113).

[9] 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 et al. (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).

probably alluding to work on photosynthesis in algae).[10] 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, the most important of which were two closely related papers, in which he dealt with the general mechanism of photosynthesis;[11] and another two papers on the efficiency of the process.[12] The advances Warburg made in these papers were enormous. He fundamentally changed the field by introducing a number of new techniques that were quickly to become standard practice in photosynthesis research and remained so until the 1970s. These included the use of manometric methods for measuring the rate and the progress of photosynthesis; and to fully exploit the advantages of this new technique, Warburg also replaced the use of leaves and whole plants with the unicellular green alga *Chlorella*, which to this day is a well-known experimental organism in photosynthesis research.[13] In addition, Warburg also employed sophisticated photophysical techniques, such as bolometry, absorption measurements and intermittent illumination by means of rotating sectors, which required not only special skills but also specific instrumentation. He was also the first to use inhibitors systematically in order to 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.[14] Finally, Warburg brought a new perspective to debates of the period by determining the energetic efficiency and, as a consequence, the minimum quantum requirement of photosynthesis—one of the few parameters at the time that limited the range of possible model alternatives. In view of the enormous impact of these new elements, it seems worthwhile to inspect them in a little more detail.

3.1.2 Manometry

Before Warburg introduced manometric methods to photosynthesis research, techniques were employed to determine gas exchanges with sensitivities measured in the

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[10] See Werner (1991, pp. 121–122). 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 later 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.

[11] Warburg (1919, 1920b).

[12] Warburg and Negelein (1922, 1923).

[13] See Zallen (1993a) for a thoughtful discussion about the use of *Chlorella* algae (and others) as experimental or even model organisms in photosynthesis research.

[14] See Warburg (1917).

range of millilitres of gas. This meant that, in order to be able to measure the minimum oxygen level in experiments, large areas of plant material had to be illuminated for a relatively long period of time, and one had to use large samples or even whole plants. Warburg's method, by contrast, offered an enormously increased sensitivity, capable of measuring *micro*litres of gas exchange.[15] Consequently, one could not only greatly reduce the sample size and the duration of experiments but also utilise smaller and more manageable light beams, all of which led to a far better control of the whole experimental set-up. The latter was Warburg's main incentive also in other situations, when he turned to designing new techniques and instruments in order to monitor biological processes. "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", Warburg maintained.[16]

Warburg had started his manometric studies with a basic manometer, which his father Emil had devised in 1900 to measure the velocity of ozonisation processes.[17] However, in March 1912, during a brief visit to the laboratory of the physiologist Sir Joseph Barcroft at the University of Cambridge(UK), Warburg became familiar with a much more sophisticated version of the instrument, which he modified even further for the analysis of either very thin slices of living tissue (for his studies in respiration) or cell suspensions (for his research in photosynthesis).[18] Figure 3.1 shows one of Warburg's own manometers, together with the specific vessel or glass flask that had to be used with it, while Fig. 3.2 is a sketch of the complete measuring device, the "Warburg apparatus".

The principal procedure was 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 thermostat (the flasks facing the interior of the thermostat, the manometer 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; in this case from below) initiated the different processes of photosynthesis, which gave rise to the evolution of molecular oxygen. The effect—namely an increase of pressure in the system—was measured by noting the changes in the height of the capillary fluid in each 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

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[15] See Myers (1974, p. 420).

[16] This was Warburg's reply, when in 1928, after a talk on the spectrophotometric analysis of the heme molecule, he was harshly criticised by Willstätter for the application of spectrophotometry to such complex structures as cells. Quoted in Nachmansohn (1972, p. 5).

[17] See Warburg (1900).

[18] A comprehensive account of Warburg's early manometric techniques can be found in his book on tumour metabolism, Warburg (1926). 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 Allen (1975, pp. 173–174).

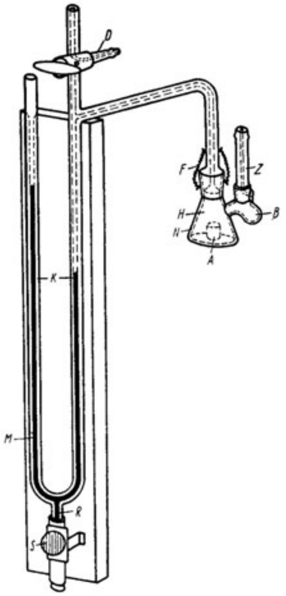

Fig. 3.1 An illustration of a manometer used by Otto Warburg in his photosynthesis experiments. (Reproduced from Warburg (1926, p. 1)).

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.

3.1.3 Chlorella

The use of manometric methods led Warburg to reconsider the test organisms suitable for the study of photosynthesis. There were some major disadvantages in 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 have several cell layers, the oxygen produced in the 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 was successful: "After some preliminary trials I kept to a round immobile green alga

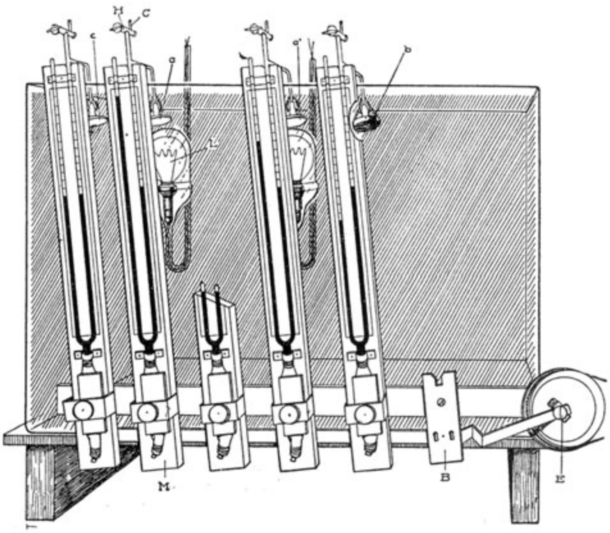

Fig. 3.2 A drawing of the complete measuring apparatus (which became known as "Warburg Apparatus"). The manometers are mounted on a thermostat, so that the vessels can be illuminated from below by light bulbs. A V-belt connected to an electric motor, part of which can be seen on the right of the illustration, oscillates the manometers. (Reproduced from Warburg (1919, p. 245)).

of 3 to 5 micrometres in diameter, which multiplies by successive fission without developing clusters or movable cells, similar to an alga described as '*Chlorella*' in the literature."[19]

As an experimental organism, *Chlorella* 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 are relatively high.[20] For Warburg, however, the main advantage was of a methodological nature. At that time, unicellular algae were the smallest organisms known that were capable of carrying out the full photosynthesis process.[21] (Chloroplasts, which would later become the preferred living structure for testing, were not isolated before the 1930s; and even then most scientists doubted whether the whole array of photosynthetic reactions could be carried out in the chloroplasts). The small size of the algae meant that the paths between the reaction

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[19] Warburg (1919, p. 231). Quoted also, originally in German, in Werner (1991, p. 148). It is highly probable that Otto Warburg was helped in his choice of organism by the phycologist Ernst Georg Pringsheim, who was one of the leading algae experts of the time. In an autobiographical account, Pringsheim reported how he was approached by Emil Warburg to participate in 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).

[20] These advantages were still emphasised by Manning (1938, p. 120), Footnote 3.

[21] See Zallen (1993b, pp. 271–273).

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 very 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 algal suspension, light readily penetrates the cells without being absorbed by non-photosynthesising regions or reflected away by a surface. Thus, using *Chlorella* enormously increased Warburg's control of the main experimental parameters, such as temperature, the gaseous and liquid environments, and light intensity.

In the years that followed, *Chlorella* became the most popular experimental organism for photosynthesis studies also in other laboratories—although there were many other species of unicellular algae that could have been used as well.[22] Two main factors accounted for this. *First*, Warburg was one of the internationally leading scientists on his field. Visitors from all over the world came to his laboratory, and then took to using *Chlorella* when they returned to their home institutions. In addition, some of the period's most influential photosynthesis researchers were trained in Warburg's institute, including Robert Emerson and Charles Stacy French, who spread the technique across the USA. *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 5 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. Its "representational scope", which Rachel Ankeny and Sabina Leonelli defined as the range of living beings for which an experimental organism was taken to be exemplary, was extremely broad—in fact, for some decades, until people became aware of the fact that there was a variety of photosynthetic pathways, it covered the whole of the plant realm. *Chlorella's* "representational target", however, that is, "the phenomena to be explored through the use of the experimental organism", mostly remained photosynthesis. Initially, *Chlorella* algae were also used for the investigation of other life processes; however, in the course of further research it became obvious that *Chlorella* algae had metabolic peculiarities that were far from generally spread even among freshwater algae. Hence, *Chlorella* never acquired the status of a "model organism", such as *Arabidopsis*, *Escherichia coli* or the mouse.[23]

Notwithstanding these difficulties, *Chlorella* has remained an experimental organism to this day—mostly because of the large amount of information collected

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[22] See on this point also Zallen (1993b).

[23] For further reflection on the representational scope and target of an organism and on the distinction between "experimental organisms" and "model organisms", see Ankeny and Leonelli (2011). The quote was taken from p. 315.

about its organismic properties.[24] Starting from the late 1930s 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 to grow the same strain of algae under the same (favourable) standard circumstances if one wanted to maintain the experimental conditions as homogenous as possible. Once appropriate culturing conditions had been defined for *Chlorella*, a task which turned out to be far from easy, many scientists were reluctant to change the experimental object again. Any modifications made, either to the conditions or to the organism itself, would have required a lengthy investigation into the comparability of the new situation with the established experimental standard.[25]

3.1.4 Buffer Solution

Using unicellular algae for manometric experiments required finding a more sophisticated solution to the problem of keeping the carbon dioxide concentration of the experimental setting at a constant value. Usually one would have turned to using carbonate–bicarbonate buffers, which were part of the standard equipment of laboratories at the time. However, because of their (slight) alkalinity, the standard buffers of this type, Warburg thought, were potentially harmful to the algae. Therefore, he developed a new 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 $NaHCO_3$).[26] It was only later, in his studies of the quantum yield of photosynthesis, that Warburg started to use acidic, phosphate-containing buffers.

3.1.5 Bolometry

In order to control the light intensities of his photosynthesis experiments, Warburg employed sophisticated bolometry, which he had learned to use in his father's

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[24] This does not, of course, mean that all the scientists working in photosynthesis research exclusively used *Chlorella*. The species of *Euglena*, *Scenedesmus* and *Porphyridium* were also used as experimental organisms, while in the field of genetic engineering *Chlamydomonas reinhardtii* became the standard; see Zallen (1993b, pp. 275 ff).

[25] On the broader question of how model organisms are being used in research, see, e.g., Creager et al. (2007): a collection of illuminating essays on the “model systems approach” as the editors call it, that is, the practice of using organisms that have become standard test objects, or of referring to case studies that have acquired exemplary status within a discipline. The editors argue that model systems, such as in this case *Chlorella*, are used as “models for” something if the system under investigation is too complex to come up with 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.

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

laboratory at the PTR. This was rather exceptional: at the time most biologists, and even chemists, were not familiar with this technique. A bolometer is a relatively sensitive device for detecting and measuring radiation intensities. The instruments in use around 1900 consisted of a Wheatstone bridge, the two branches of which were connected to very thin (0.0025 mm) strips of metal (e.g. steel, platinum, palladium), a battery and a galvanometer for measuring electrical currents. When one of the two metal strips was exposed to radiation, the metal heated up, which increased its electrical resistance. Consequently, the galvanometer could detect a certain voltage between the two parts of the system, proportional to the amount of radiation energy incident on the metal. Emil Warburg improved the device further when he developed the vacuum bolometer that was mentioned earlier. By 1907, bolometers could thus detect temperature changes of 0.00001°C—which, of course, exceeded by far the degree of precision that was sensible to ask for when working with living organisms.[27]

3.1.6 *Rotating Sectors: The Flashing Light Technique*

A second non-standard technique (for biological experiments) that Warburg also had got to know through his father was the use of "rotating sectors" and, hence, intermittent illumination. To this effect, 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 (see Fig. 3.3). Rotating sectors were standard instruments in the field of photophysics and, therefore, regularly used in the optical laboratory of the PTR, where Warburg carried out many of his early experiments in photosynthesis.[28] This technique enabled Warburg to study photosynthesis under conditions in which the light reactions limited the velocity of the process, as will become clear in a later section of this chapter.

3.1.7 *Inhibitors*

Warburg was the first to use biological inhibitors as a means to find out more about the biochemical mechanism of photosynthesis. In particular, he investigated the effect of anaesthetics, such as the different urethanes, and of hydrogen cyanide. Warburg already knew the inhibiting effect of these substances from his studies in respiration:

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[27] See Warburg et al. (1907) for the description of the vacuum bolometer; cf. also the popular account of this instrument, at Anonymous (1907b).

[28] See Warburg (1919, pp. 235 and 255). Warburg also seems to have been inspired to use rotating sectors by the work of the English plant physiologist Horace Brown and his collaborator Francis Escombe; see Brown and Escombe (1905, p. 38). Warburg acknowledges Brown's influence in Warburg (1919, p. 263).

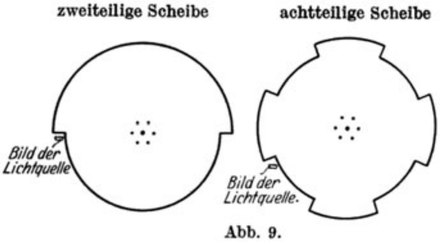

Fig. 3.3 A drawing of Warburg's rotating sectors in Warburg (1919, p. 251), Fig. 9. Two different variants are depicted: on the left, a disc divided into two halves (i.e., two periods: one dark, one light) is shown, while the disc on the right is divided into eight sections (resulting in four dark and four light periods each). The tiny rectangle to the left of each disc marks the position of the light source (*Bild der Lichtquelle*).

urethanes were known to inhibit processes dependent on internal cell surfaces, while hydrogen cyanide blocked the haemoglobin's site of oxygen binding (at the iron component). If the process of photosynthesis, or some of its components, were also inhibited by these substances, one could then draw inferences on the properties of some necessary factors and, hence, on the way the process functions.

3.2 Warburg's Early Photosynthesis Model

Warburg's first two articles were 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] His goal was to ascertain "by which means those substances that take part in the assimilation process are rendered reactive in living cells".[30] Why was it, Warburg asked, that, in the process of photosynthesis carbon dioxide decomposes, although under normal circumstances (notably at room temperature) it is usually almost completely inert? His explanation was, in short, that the reactivity was increased by the substances involved 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. Warburg postulated that three different classes of reaction were involved:

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

[30] See Warburg (1921, p. 354). Original German text: "... wodurch die an dem Assimilationsvorgang beteiligten Stoffe in der lebenden Zelle reaktionsfähig werden".

(i) A primary photochemical process of light acting on 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 the following sections, I shall present Warburg’s main evidence for these hypotheses and reconstruct the course of his argument—unfortunately, no laboratory notes of Warburg survived for that period of his research, so that the following is based on his publications.[31] The model that Warburg developed was not to last for long, yet his argumentation deserves attention for two reasons: *first*, it impressively demonstrates the difficulties that biochemists and cell physiologists at the time were struggling with, as the body of data to infer the course of internal processes was so meagre and only allowed for indirect conclusions. At the same time, *second*, it equally impressively demonstrates the ingenuity of Warburg’s work, both in terms of experimentation and interpretation. Warburg really squeezed out as much as possible from the little evidence he had and argued for the legitimacy of every single step—hence, his argument provides an excellent example of the construction of a complex model hypothesis. In order to explain Warburg’s inferences from his data—and to appreciate his approach to the problem which fundamentally differed from the chemists’ work presented in chapter 2—some technical detail is required, while a more approachable summary is given in section 3.2.2.

3.2.1 Experimental Findings and the Interpretations thereof

3.2.1.1 Carbon Dioxide Concentrations

Warburg began his work in photosynthesis by re-examining the standard parameters of photosynthesis as investigated thus far by plant physiologists—this was inevitable, Warburg explained, as his predecessors had been using unsatisfactory techniques and instruments. (None of the chemists mentioned so far had ever attempted to repeat—and improve upon—the plant physiologists’ work; while, even if they had considered this option, they would not have had the methodical skills to do so.) The first theme

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[31] A first account was given in Nickelsen (2007).

Warburg revisited was the relationship between photosynthesis and the levels of carbon dioxide concentration, measured at high light intensities. There were no surprises here: Warburg confirmed the findings of the English plant physiologist Frederick F. Blackman and his collaborators, who in 1905 had established the fundamental Law of Limiting Factors, a reformulation of Justus Liebig's Law of the Minimum. This so-called "law" stated that it was not the totality of resources that limited the rate of a chemical reaction (or of a physiological process such as growth) but the availability of the scarcest factor.[32] 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 an increase in the rate of photosynthesis, 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. Since light intensity and temperature were chosen favourably, he thought, 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. 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.[33] 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 reconstruction of the sequence of events that Warburg proposed is shown in Fig. 3.4.

3.2.1.2 Light Intensity

The second issue that Warburg re-examined was the relationship between photosynthesis and light intensity, measured at high carbon dioxide concentrations. He found that 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 familiar (although Warburg's new technique produced a slightly different curve), but Warburg proposed his own interpretation, while he emphasised the similarity of this effect to the one described above:

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[32] See Blackman (1905). Blackman was the first to investigate, together with various collaborators, the influence of several parameters on the rate of photosynthesis, including light intensity, temperature and carbon dioxide concentrations.

[33] Warburg (1919, p. 253): "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" (author's translation). Warburg cites Willstätter and Stoll (1918, p. 172, 226 ff.)

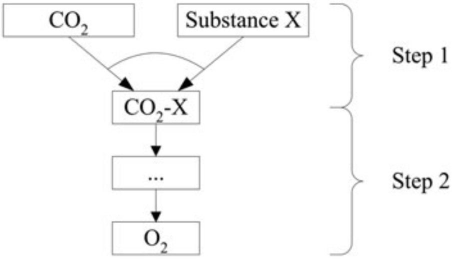

Fig. 3.4 Warburg's interpretation of the carbon dioxide curve. In the first part of the curve, CO_2 itself would be limiting the process, while a second substance, X, was thought to be the limiting factor in the second part of the curve, so that no additional increase in carbon dioxide concentrations 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.

> 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 agreement 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.[34]

Thus, Warburg thought that also 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.[35] This was the first time that the shape of this light intensity 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: In the first stage light reacts with some other substance, Z, to form 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 (see Fig. 3.5).

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[34] Warburg (1919, pp. 257–258).

[35] 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. See Warburg (1920b, pp. 210–211).

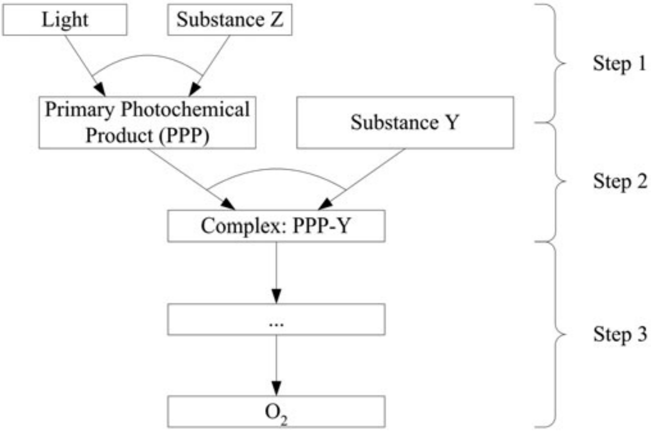

Fig. 3.5 Warburg's interpretation of the light curve. The first step consists of a primary photochemical reaction of light with substance Z, resulting in the primary photochemical product (Step 1: PPP). This product immediately undergoes a reaction with a second substance, Y, and a complex of PPP and Y is formed (Step 2: PPP-Y). The latter is then subject to further reaction steps leading to the release of oxygen (Step 3).

3.2.1.3 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 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.[36] 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 4–5, 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 was governed by a process that is practically temperature independent: a photochemical reaction was the obvious answer.[37]

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[36] Warburg (1919, p. 258).

[37] 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 temperature decreased. This, Warburg argued, indicated that in this process high energy substances, such as PPPs, were the limiting factor. Warburg (1921, p. 355).

3.2.1.4 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. 3.3). 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.

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 assumed that during these "dark" processes a substance was formed that would 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 interpretation of the light intensity curve: the light-dependent reaction, the primary photochemical process, provided only part of the necessary raw materials for the eventual release of oxygen. The other component was supposed to be an additional substance, Y, which had to react with the PPP (see above and Fig. 3.5). 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. 3.6), for the extended model).

3.2.1.5 Anaesthetics

The effect of inhibiting substances, especially anaesthetics, on photosynthesis played an important role in Warburg's reasoning (see above). He predominantly investigated the effect of urethanes, in particular phenylurethane, which was known to reversibly inhibit life processes. 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. 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

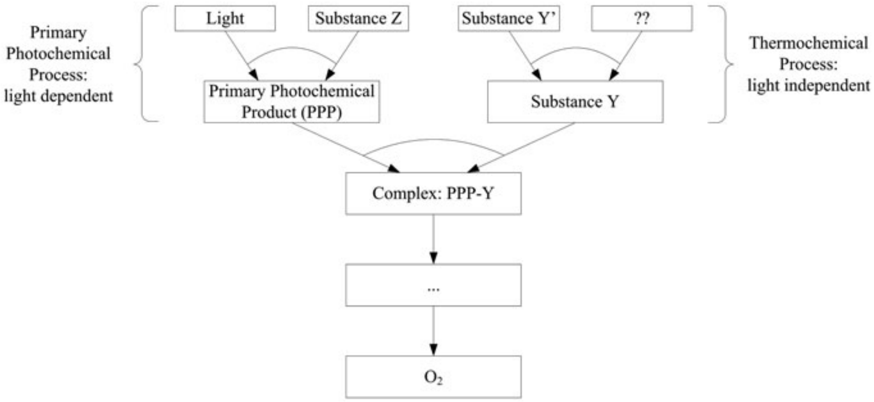

Fig. 3.6 The extended model of Warburg's interpretation of the light curve: whereas the PPP is formed during a light-dependent process, substance Y is produced during a light-independent series of reactions. The former limits the rate of photosynthesis at low light intensities, the latter at high light intensities.

> processes, as, for example, respiration and fermentation, the slightest mechanical change to the cell structure will suspend [photosynthetic] assimilation.[38]

This interpretation matched Warburg's earlier finding that the inhibiting effect of an anaesthetic substance was proportional to its adsorptive capacity, that is, its tendency to adhere to surfaces.[39] 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—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 a reaction that took place on the cell's internal surfaces, presumably on the surface of the membranes.[40] 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. As the absorption of light itself was surely not sensitive to anaesthetics, Warburg concluded that a secondary (although indispensable) surface-sensitive reaction must take place. This corresponded well to

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

[39] Warburg (1920b, pp. 196–197).

[40] Warburg (1920b, pp. 197–199).

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.

3.2.1.6 Hydrogen Cyanide

In addition, Warburg examined the influence of hydrogen cyanide, another substance with inhibiting effects, albeit for fundamentally different reasons. 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.[41] 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. Warburg, thus, suggested that hydrogen cyanine inhibited "the ability of carbonic acid to undergo photochemical reactions".[42] 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 this binding process that Warburg thought would be inhibited by hydrogen cyanide. From other contexts, it was known 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, hence, inferred that the reaction in question was an enzyme-catalysed reaction requiring the involvement of heavy metals.

3.2.1.7 Photochemical Induction

Finally, Warburg investigated 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. 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 by secondary reactions of this chain reaction process rather than by the primary photochemical reaction.[43] A similar phenomenon, Warburg argued, could also be observed in photosynthesis, when studied under intermittent illumination. Only after some minutes of illumination, Warburg reported, would the usual constant value of carbonic acid decomposition be reached. However, this was only the case at high light intensities, as he could not demonstrate any such delay at low light intensities. 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

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[41] Warburg (1919, p. 266).

[42] Warburg (1920b, p. 199).

[43] See Warburg (1920b, p. 189).

> 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.[44]

3.2.2 Photosynthesis Framed as Photolysis

Warburg integrated all these findings into a model of the mechanism of photosynthesis, which is reconstructed in a graph form in Fig. 3.7. Warburg had investigated the different partial aspects of the mechanism of photosynthesis more comprehensively than anybody before him and had tried to collect quantitative data on every single step of the process. Yet, the final model of this mechanism still comprised conceptual steps that went beyond these data. 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."[45] The latter, that is, the constituents of the photolyte which react with PPPs, would be called "acceptors". (This, of course, fundamentally differs from what today is called an "acceptor" in photosynthesis, mostly used in the context of either electron or hydrogen acceptors in the electron transport chain). However, as Warburg stressed, photosynthetic assimilation was "not a simple photolysis of carbonic acid":

> The primary photochemical process, during which oxygen is 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

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[44] Warburg (1920b, pp. 208–209).

[45] Warburg (1920b, p. 206).

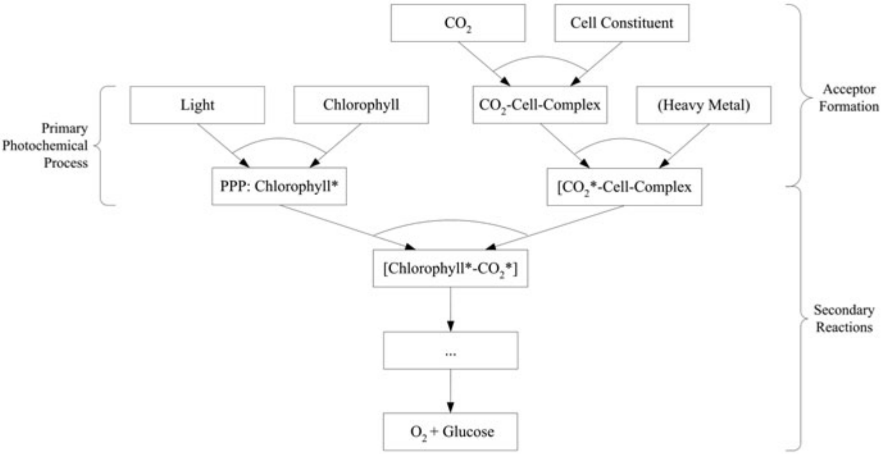

Fig. 3.7 A reconstruction of Warburg's photosynthesis model.

> the secondary reaction, which destabilises the dark equilibrium.l Both the reactions that lead to the formation of the acceptor and the reaction between the acceptor and the primary photochemical product are 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.[46]

This was the core of Warburg's photosynthesis model. The primary process, as Warburg underlined, was the most elusive reaction of the whole mechanism.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.[47] On absorbing light energy, the short-lived PPP is formed, which in 1921 Warburg assumed to be the "isomers of the [light absorbing] pigments, enriched in energy by hν".[48] The higher energy level of chlorophyll in this activated state is indicated in the figure by an asterisk (*). 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.[49]

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[46] Warburg (1920b, pp. 206–207).

[47] To simplify matters, the Fig. 3.7 includes only the chlorophyll molecule, although Warburg acknowledged that in addition to the two kinds of chlorophyll (*a* and *b*), also the xanthophylls and the carotenes contributed to light absorption.

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

[49] Again, it is important to keep in mind that the current usage of "acceptor" does not correspond to Warburg's notion of the term!

Because of this sequence 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.[50] 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 bound carbonic acid. Since this partial process had proven itself 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 Fig. 3.7 by an asterisk [*]). Furthermore, it was also shown to be surface dependent, given 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, whereby the carbonic acid derivative was reduced. Warburg did not go into much detail here, except to characterise these reactions again as surface-dependent, purely chemical processes.

3.3 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-authored with his long-standing collaborator Erwin Negelein. 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?"[51] 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 the "specific photochemical effect" (*spezifische photochemische Wirkung*), abbreviated to φ, which denoted the chemical work effected by one calorie of absorbed

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[50] Warburg (1921, p. 355). However, in 1925 Warburg 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).

[51] Warburg and Negelein (1922, p. 235).

radiation.[52] 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.[53]

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, where they used the institute's high-quality area bolometer.[54] Warburg and Negelein exposed *Chlorella* to light of wavelengths between 570 and 645 nm, that is, from yellow to orange light. In order to get a reliable value for the amount of absorbed energy, E, Warburg and Negelein used very thick algal suspensions, so that practically all the incident light on the sample was absorbed. By contrast, the chemical work 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. The efficiency measurements themselves revealed that, on average, an extremely high percentage of between 60 and 70 % of the absorbed radiation energy could be transformed into chemical energy—perhaps even more. This was spectacular, given that 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 %![55]

Although the maximum quantum yield of photosynthesis had occasionally been discussed in the years before Warburg and Negelein turned to the subject, it was far from the "frequently debated" issue that the authors claimed it to be in their introduction. In fact, a review of 1916 had seen a definite need for more research on the "energy relations of the green leaf", to which was added: "This aspect of carbon assimilation exhibits perhaps more than any other an unfortunate isolation of effort in research, the various workers on the subject having generally neglected the results

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[52] See Warburg (1920a, p. 54).

[53] Warburg and Negelein (1923, p. 205).

[54] Warburg and Negelein (1922, p. 236).

[55] In the second publication of 1923, the average value of 1922 (70 %) was slightly reduced to an average (in red light) of 59 % efficiency, while the maximum value they had been able to achieve was 63.5 % 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 and Negelein (1923, p. 205).

obtained by others, both along their own and related lines of investigation".[56] Measuring quantum yields certainly occupied the attention of photochemists; but very few people had so far tried to transfer this approach to the study of photosynthesis. The standard estimation had been provided in 1905 by the English plant physiologist Horace Brown and his collaborator Francis Escombe, who had found a maximum efficiency of photosynthesis of no more than 6 %, that is, a fraction of what Warburg and Negelein claimed to be the case. Warburg and Negelein argued that Brown and Escombe's results were invalid: They 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.[57]

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 of 49 kcal each. Although Warburg and Negelein did not yet dare, in 1922, to draw any concrete inferences from their findings, they did emphasise that in view of the high overall efficiency of the process, the reduction of carbonic acid had to be rather straightforward, that is, without the inclusion of high-energy intermediate reactions.

These general findings were followed in 1923 by an investigation into the influence of different wavelengths on the efficiency of photosynthesis.[58] 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 agreed 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 5 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 20 years or so; and it was these figures that sparked off the vigorous controversy on quantum yields and efficiencies between Warburg and the American photosynthesis researchers. This controversy is the subject of chapter 5.[59]

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[56] Jörgensen and Stiles (1916b, p. 24).

[57] See Warburg and Negelein (1923, pp. 192–193).

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

[59] See on this episode also Nickelsen and Govindjee (2011).

3.4 Father, Son and Photosynthesis

Considering the analysis of Warburg's early photosynthesis work from a slightly different perspective, I would like to draw attention to the following three points: (1) Warburg explicitly adopted theoretical concepts—photolysis, the photolyte and the photochemical yield—that his father had used and developed before him; (2) Warburg undertook experiments along the lines of his father's earlier work and stressed that his findings concurred with his father's results; (3) 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, it seems that the connection between Otto Warburg's research and the studies of his father Emil deserves some special attention.

3.4.1 *Emil Warburg and Photochemistry*

As is well-known, the PTR, to which Emil Warburg was appointed its president in 1905, was a national research institution primarily concerned with careful measurements and the definition of standards.[60] The radiation laboratory, in particular, had a renowned history and a high international reputation. It was in this highly equipped laboratory that the physicist Otto Lummer and his collaborators had started to do research on 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 had left the PTR in 1904 to take up a professorship at the University of Breslau (today's Wrocław), this instrumentation fell into disuse.[61]

The revival of the radiation laboratory is today considered one of Emil Warburg's most notable achievements as President of the PTR. Already shortly after having taken up this position, Emil Warburg started investigating the energetics of photochemical processes, while after 1911 he made it the focus of his work. Between 1911 and 1919, he published nine important articles on photochemistry, a subject that clearly dominated the latter part of his career.[62] 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 sparked by Einstein's seminal publication on the light quantum hypothesis (1905), in which Einstein had postulated that: (1) radiation of

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[60] See Cahan (1989), which provides an account of the PTR's history, 1871–1918.

[61] Lummer's rotating sectors were used later by Emil Warburg to determine the constant c of the black body radiation law and, eventually, by Otto Warburg for his photosynthesis studies. See on Emil Warburg's work the report on pp. 118–120 in: Tätigkeitsbericht der PTR für das Jahr 1910, *Zeitschrift für Instrumentenkunde*: Heft 4, pp. 106–120; Heft 5, pp. 140–160; Heft 6, pp. 174–195.

[62] On Emil Warburg's photochemical work, see, e.g., Franck (1926, 1931). Brodhun (1913) provides a detailed review of the PTR's then recent activities in optics.

the frequency ν consists of discrete light quanta of the energy h (Planck's constant) times ν; and that (2) matter that absorbs (or emits) radiation will do so in terms of light quanta of this type.[63] Although this hypothesis had many explanatory virtues, it was far from complete and the exploration of its consequences gave rise to a wealth of puzzles to be solved.

Although the role of light in chemical processes had been much debated already in the nineteenth century, the quantitative analysis of these phenomena had remained a problem.[64] On the one hand, methodical difficulties impeded the research, since photometry was still in its infancy around 1900. On the other hand, conceptual problems prevailed. In 1909, Einstein himself summarised one of the main inconsistencies 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?"[65] It did not take Einstein long to answer these questions. 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."[66] At the same time, Emil Warburg had turned to these questions, which he approached from an experimental perspective. He had even devised a new type of bolometer for this project, the vacuum bolometer, which allowed for more precise measurements to be taken.[67]

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 this discussion that brought Einstein to formulate the aforementioned Law of Photochemical Equivalence.[68] One 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

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[63] See Einstein (1905) for the original publication; The paper was reprinted (together with an introduction and additional notes) in Vol. 2 of the *Collected Papers* edition; see Stachel et al. (1989), pp. 134–169.

[64] See the introduction to Einstein's paper in Klein et al. (1995, pp. 109–111). For a review of the problem, see Warburg (1917). See also Boberlin (1993) for an account of the nineteenth-century beginnings of quantitative photochemistry.

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

[66] See Klein et al. (1995), introduction, p. xiv. For Einstein's first paper on the subject, see Einstein (1912b), which is reprinted in Klein et al. (1995), Doc. 2, pp. 115–121. For a more detailed study of Einstein's work on photodecomposition, see Bergia and Navarro (1988).

[67] See Warburg et al. (1907) and Warburg (1909) for the announcement of the instrument, while in Warburg (1912, 1913) he presented the first results of the project.

[68] 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 et al. (1993), Doc. 309, pp. 352–353: "I also have an issue with Warburg, who was in Brussels. He has proven, wrongly, that there must be a threshold for photochemical excitation. On this occasion I found an interesting thermodynamical proof for the Law of Photochemical Equivalence that Warburg seeks to confirm. 1 molecule is dissociated by ν-radiation at the absorption of an energy hν." (Translated by the author).

was able to undergo photochemical cleavage.[69] Emil Warburg then 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.[70] This work was facilitated by the fact that Einstein had moved to Berlin in 1914 and had been able to keep in close contact with Emil Warburg since.[71] In 1915 Einstein even 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). Einstein also 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.[72] 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.[73]

3.4.2 *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 defined by his father in the latter's work on photochemistry; *second*, Otto Warburg applied some of his father's photochemical concepts—the "photolyte" and the notion of "photochemical efficiency"—to his own work. *Third*, thanks to his father's position, not only was Otto Warburg able to use the excellent optical instruments at the PTR but was also introduced to photochemical experimentation by the experts in the field as well as given 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 1905–1906 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. Starting from

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[69] See Einstein (1912a, 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.

[70] See Warburg (1917, 1924)..

[71] In fact, Emil Warburg had been one of the members of the Berlin Academy of Sciences and Humanities who had been behind Einstein's appointment; see Goenner and Castagnetti (2004) and Goenner (2005).

[72] See, on this point, Einstein's letter to Otto Warburg of 23 March 1918, urging him to return to Berlin from the war, in which Einstein wrote: "You will probably be rather surprised to receive a letter from me, for until now we have only circled each other without ever getting truly acquainted"; Schulmann et al. (1998), No. 491 (originally German, translation by the author).

[73] The outcome of one of these occasions was the employment of Hans Krebs in Warburg's laboratories in 1926, as Krebs himself reported. Quoted in Werner (1991, p. 137), doc. 45.

1918, the experiments that Otto Warburg carried out at the PTR were documented in the institution's yearly reports, and in his papers Otto Warburg duly gave credit to the support given to him. He even expresses gratitude to the staff for having carried out measurements on his behalf.[74] 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 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 description of this project's aims:

> 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 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.[75]

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.[76] 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 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[1]—which, by the way, otherwise contains little of interest—that two Englishmen, Brown & Escombe (Int. Trans. Roy. Soc. 183 B 223, 1900; Proc. Soc. 76 B, 1905; Nature, March 1905) have 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.
>
> [1] [Footnote:] *ZS für wissenschaftliche Photographie, Photophysik & Photochemie.* Vol. 11, issue 2, p. 381.[77]

The letter is particularly interesting as it refers to experiments that father and son obviously were intending to carry out together. The paper of 1905 that Emil Warburg mentioned is the very article written by Brown and Escombe that Warburg later

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[74] See Warburg (1919, pp. 235, 255).

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

[76] 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., the extensive treatment of photosynthesis in Fritz Weigert's monograph on the chemical effects of light, Weigert (1911).

[77] 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.

dismissed as being based on methodically flawed experimentation.[78] Thus, although Otto Warburg only published his first article on photosynthesis in 1919, his father already had been planning to work with him on this theme as early as 1912. One could speculate that they might have discussed this option 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 still deeply involved in the study of respiration, and the planned experiments had to wait.

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 subject had advanced (if at all) when Otto Warburg enlisted for service in the First World War. All we know is that neither Emil Warburg nor Carl Correns hesitated to mention photosynthesis experiments as an argument for calling Otto Warburg back to Berlin. And, 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:

> 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.[79]

According to the 1919/1920 report of the KWI, photosynthesis was also Warburg's main research theme in the following year.[80] Thus, on closer investigation, one finds evidence that Otto Warburg, together with his father, was interested in photosynthesis as early as 1912. And although conclusive evidence for Otto's research into this theme is available only for the time after 1918, it is highly probable that this work was influenced, and promoted, by Emil Warburg's earlier research aims, formulated in 1911, and the PTR's facilities. Yet, Otto Warburg did not immediately turn to the reaction's efficiency or its quantum requirement, which was in the centre of his father's interest, but instead presented a comprehensive investigation of the chemical mechanism of photosynthesis. And although Otto Warburg made ample use of his father's methods and concepts therein, an even more important source of motivation, not only for the theme in general but also for its specific treatment, emerges if one takes a closer look at Otto Warburg's research up until 1914, in particular his studies in cell respiration.

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[78] The references cited by Emil Warburg are Brown and Escombe (1900, 1905); Brown (1905) and Weigert (1912).

[79] Translated from Werner (1991, p. 128). During the war, the rooms of Warburg's laboratory had been used by a team led by Fritz Haber to conduct experiments 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.

[80] Werner (1991, p. 146).

3.5 Studies in Cell Respiration

Otto Warburg's research into cell respiration—or biological oxidation, which is the more general term—has already been the subject of a number of excellent studies.[81] It was for this field of research that Warburg was most famous and for which he received the Nobel Prize in Physiology or Medicine in 1931. However, this section focuses on the early years of Warburg's research, that is, the period 1908–1914, which means that Warburg's final success, his concept of *Atmungsferment*, which was developed in the 1920s, has been omitted from the discussion.[82]

Cell respiration was the first theme that the young Warburg chose to study independently, making it the topic of both his medical dissertation in 1911 and his habilitation in 1912. Warburg's work in these years was strongly influenced by the physiologist Jacques Loeb, then based at the Rockefeller Institute for Medical Research in New York, and his "mechanistic conception of life": the programme to explain life processes in a physico-chemical way.[83] Warburg frequently went to the renowned Zoological Station at Naples (Italy), a research institute that developed into a hub for biological research, where he met the avant-garde of this new research tradition, which included Theodor Boveri, Hans Driesch, Oscar Hertwig and Thomas H. Morgan.[84] However, although Warburg received much inspiration from their work on developmental physiology, he quickly developed his own agenda, which was mainly concerned with the physico-chemical elucidation of energy-producing reactions: a subject that was then extremely controversial.[85]

As later with photosynthesis, Warburg also fundamentally changed research in the field of cell respiration research by introducing both new techniques and new conceptual approaches.[86] Warburg was, for example, the first to study this process in isolated cells. Up to then the experimental organisms used for this purpose had been mice, rabbits and other animals, which made it extremely difficult to control the experimental conditions. Warburg rather chose to investigate sea urchin eggs, which were then the main experimental objects employed in the field of developmental

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[81] For Otto Warburg's early concept of the *Atmungsferment*, see, in particular, the thorough study by Kohler (1973a). Höxtermann (2007a) provides a complementary explanation of Warburg's understanding of biocatalysis. The latter is also treated in Höxtermann (2001, pp. 265–268) and in Werner (1991, pp. 64–69, 113–118). The introduction of Werner (1996) provides rich historical background to the theories of respiration at the time, while Werner (1997) analyses the controversy that arose between Warburg and Heinrich Wieland on the theory of biological oxidation. More specific references can be found in the above-mentioned publications.

[82] I gratefully acknowledge conversations with Ekkehard Höxtermann, Berlin, on this theme, which helped me improve this section.

[83] Cf. Loeb (1905). On Loeb's influence on Warburg's research, see Werner (1996, pp. 35–47).

[84] See on the *Stazione Zoologica di Napoli*, e.g., Groeben (1975); Fantini (2002) and Groeben (2005). A brief overview is given in Nickelsen (2010). Dohrn (1892) provides a contemporary perspective; see also the excellent collection of essays in Metz and Clapp (1985).

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

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

biology. Indeed, it is very likely that also Warburg originally intended to study the problems of embryonic development: in his first publication on the subject, he announced further investigations into the early cleavages of the fertilised egg. The rate of oxidation was only the first parameter to which he turned.[87] In other words, studying the respiratory processes of the fertilised egg initially was only a subgoal, while his actual, superordinate goal was to understand the underlying chemical mechanisms of early embryonic cell cleavage. Yet, the subgoal turned out to be so interesting that Warburg very quickly made it the main focus of his further research.

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. Far more important were methodological considerations: Since it is easier to carry out controlled experiments on simple systems, the preferred choice for quantitative studies were the smallest possible units (we can recognise the similarities to his later choice of *Chlorella* in photosynthesis research).[88] It was also during the course of his studies in cell respiration that Warburg developed his sophisticated technique of manometry by adjusting the manometers that Barcroft had used (see above) to the requirements of his own project. Warburg then explored new ways of using the reversible inhibition of cell processes by 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.[89] Thus, a large part of the equipment and substances that Warburg would later so innovatively introduce to photosynthesis research had originally been developed in his earlier studies in cell respiration: the use of single cells as the experimental 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 papers on cell respiration 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?[90] Some sort of catalysis, it seemed, had to be involved; but then, how should this catalysis be described? Following the discovery of "zymase" (an intracellular enzyme complex) by the German chemist Eduard Buchner in 1897, two options presented themselves: the decisive factor was either the action of the cell structure (which was the biologists' view) or the action of

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[87] Warburg (1908, p. 1).

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

[89] 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).

[90] Warburg (1914, p. 314).

an enzyme (which was the chemists' view). Warburg solved this issue by integrating both modes of catalysis into one complex mechanism, as he 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*".[91]

Through his use of surface-active inhibitors, Warburg was able to establish over the course of the years that internal cell surfaces were, for the most part, 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 conception 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".[92] 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. 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, called X, which was the first substance to be oxidised. However, in a review article of 1914, Warburg suggested that the relevant mechanism included the "oxidation of lipoids in the presence of iron salt".[93] Warburg concluded this from his experiments with lecithin, which he seems to have taken to be representative of the whole group of lipoids. It was known that the internal cell structures that Warburg considered so important were, in large part, made up of lipoids. In Warburg's model of 1914, these 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. 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

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[91] Warburg in 1914; translation taken from Kohler (1973a, p. 190). Emphasis in the original.

[92] Höxtermann (2007a, pp. 123–124). For the complicated development of the concepts of "enzyme" and "ferment", see, e.g., Fruton (1972b); Kohler (1973b) and Teich (1981).

[93] Warburg (1914, p. 335).

early as 1914, Warburg was able to present an impressively detailed account of cell respiration, based on rich empirical evidence.

The similarities between this model and Warburg's photosynthesis model of 1919 are obvious. 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. In his 1927 book on the catalytic action of the living substance, a collection of selected papers 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.[94]

The similarities in his conceptions of respiration and photosynthesis were no coincidence. Warburg was thoroughly convinced that the same 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. In her diaries, Warburg's sister, Lotte, wrote that, in 1926, Warburg commented on Carl Correns, who was irritated by the fact that, at the time, 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".[95]

Given this attitude and Warburg's interest in energy-producing reactions (not only in respiration but also in fermentation), his shift to studying photosynthesis is no longer surprising. It rather is another prime example of research opportunism in action. Warburg had been highly successful in elucidating the mechanism of cell respiration: why not examine the second large class of energy-producing reactions, namely photosynthesis, with the same approaches and see whether similar principles held there? From this perspective, it is obvious why Warburg at first turned to the photosynthesis mechanism (which he was able to study with the methods at hand), while only later he decided to study the subject from the point of view of energetics, which also interested his father. Finally, the fact that Warburg had used sea urchin eggs for his studies in cell respiration explains why he did not continue this line of research immediately after 1918, although in 1914 his work was looking very promising. Warburg had either worked in Naples or had sea urchins from the Zoological Station sent to Berlin. Neither of these options prevailed any longer after the war: "The more our currency goes down, the farther away is Naples", Warburg wrote in 1922 in a letter to Reinhard Dohrn, who was then the head of the Naples institution.[96]

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[94] Warburg (1927, p. 12).

[95] See Rüskamp (1989, p. 252); also quoted in Werner (1991, p. 143).

[96] Quoted in Werner (1991, p. 130); translated by the author.

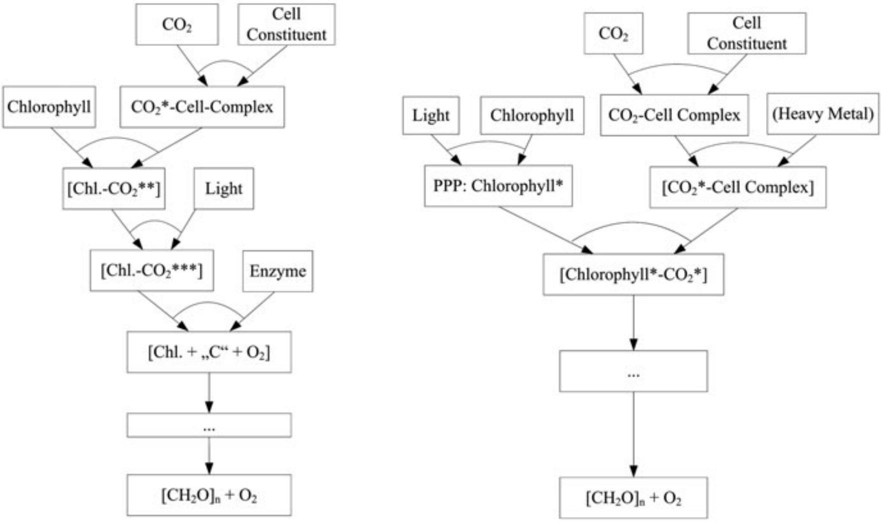

Fig. 3.8 A comparison between the photosynthesis models of, *left*, Willstätter and Stoll (1918) and, *right*, Warburg (1919, 1920).

Thus, Warburg was forced to become involved with research questions that could be carried out using more mundane organisms—such as freshwater algae, which could be retrieved from the nearby Schlachtensee.

To sum up: there is much evidence to support the assumption that Warburg started to contribute to photosynthesis research not because his father prompted him to do so, but mainly because the opportunity arose and he had the means to do so. Perhaps Warburg only chose to embark on this theme 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 sea urchin eggs. Warburg then found, however, that with his approaches and techniques he could, in actual fact, make a contribution to the subject; yet, very soon thereafter, he rather turned to the study of cancer. The year 1925 may 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 made Warburg focus his attention on these questions again.

3.6 Comparison with the Chlorophyll-Complex Model

To round off the analysis, I shall now briefly discuss how Warburg's model relates to the lines of research that were followed in chapter 2. In Fig. 3.8, Warburg's proposal has been juxtaposed with the chlorophyll-complex model of Willstätter and Stoll, which was published in 1918 and, as was previously mentioned, was considered to be the most satisfactory photosynthesis model that had so far been developed. 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, he may 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).

Warburg had evidence, for example, that a surface-dependent thermochemical reaction occurred that was sensitive to hydrogen cyanide (which implied that the reaction in question most probably involved the action of heavy metals). Kinetic data also suggested that carbon dioxide (or 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 and Stoll 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.[97] 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 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 experimental 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 seldom regarded 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 factors postulated in the chlorophyll-complex model of Willstätter and 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".

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[97] See Höxtermann (2007a) 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.

3.7 Otto Warburg's 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—in line with the terminology of Frederick L. Holmes one might speak of a very plausible twist in Warburg's "investigative pathway".[98] Early in his career Warburg chose to focus 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 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 line of research 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. However, as a physicist, Emil Warburg felt that he could not deal with living organisms, so that he tried to convince his son to collaborate with him (after Pringsheim had declined to join this project). 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 can be attributed to the work that he carried out on cell respiration while in his late twenties. He continued to use manometry as a measuring technique and single cells as experimental 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. Hence, he exemplifies a knowledge transfer that went far beyond Lindley Darden's transfer of (conceptual) mechanism schemes. 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 approach that was introduced in chapter 2. Warburg used techniques, instruments and concepts that he had acquired from his own experience as well as from other scientists, such as (1) the standard

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[98] Holmes (2004).

body of knowledge of the time; (2) Warburg's own, earlier achievements, 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.

3.8 Warburg's Impact on Photosynthesis Research

Even though Warburg may have come to study photosynthesis partly by chance, in some ways he provided exactly what the field needed at the time. The extensive review of 1916 by Jörgensen and Stiles that has been cited earlier offered in its final section a vision for the further development of photosynthesis studies:

> This is the prospect that plant physiology is developing into an exact science, utilising the experiences of the fundamental sciences, physics and chemistry, but nevertheless a science, exact and independent, with its own working principles and methods, directing and stimulating the development of the applied sciences, agriculture and horticulture. [...] It is clear that the only way to attain a reasonable rate of progress is to institute a much closer and more intimate cooperation between scientific workers attacking the same problems from different points of view and by different methods.[99]

While Warburg was not the right person to institutionalise cooperation (he always preferred to work by himself), he was one of the few people at the time who combined practical and theoretical expertise from physics, chemistry and physiology. In order to find the photosynthetic mechanism 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 a novelty at this time), Warburg ingeniously utilised the progress made in the basic concepts of chemistry. This interdisciplinary mixture of approaches and techniques would soon become characteristic of the field.

The extent to which Warburg influenced photosynthesis research cannot be overestimated. Manometric studies provided information on the chemical and physical details of photosynthesis, which up to then had not been obtainable. The pertinent techniques very soon dominated the field, and by the 1930s the fruitless search for chemical intermediates had largely been dropped. Kinetic studies using gas exchange measurements became the standard approach—at least in those institutions that specialised in photosynthesis research. 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,

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[99] Jörgensen and Stiles (1916b, p. 91).

and even a decade after Warburg's first papers had been published, the use of unicellular algae as experimental organisms remained unfamiliar to most botanists.[100] Warburg's (and Willstätter's) findings and particularly their techniques only gradually found their way into university curricula. Photosynthesis remained a side issue, a situation that would not change until after 1945.

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[100] See Pirson (1994).