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LETTER TO THE EDITOR

Three overlooked photosynthesis papers of Otto Warburg (1883–1970), published in the 1940s in German and in Russian, on light-driven water oxidation coupled to benzoquinone reduction

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Abstract
After a brief background on Otto Heinrich Warburg (1883–1970), and some of his selected research, we provide highlights, in English, of three of his papers in the 1940s—unknown to many as they were not originally published in English. They are: two brief reports on Photosynthesis, with Wilhelm Lüttgens, originally published in German, in 1944: 'Experiment on assimilation of carbonic acid'; and 'Further experiments on carbon dioxide assimilation'. This is followed by a regular paper, originally published in Russian, in 1946: 'The photochemical reduction of quinone in green granules'. Since the 1944 reports discussed here are very short, their translations are included in the Appendix, but that of the 1946 paper is provided in the Supplementary Material. In all three reports, Warburg provides the first evidence for and elaborates on light-driven water oxidation coupled to reduction of added benzoquinone. These largely overlooked studies of Warburg are in stark contrast to Warburg's well-known error in assigning the origin of the photosynthetically formed dioxygen to carbonate.

"Truth is more likely to come out of error if it is clear and definite, than out of confusion, and my experience teaches me that it is better to hold an understood and intelligible opinion, even if it should turn out to be wrong, than to be content with a muddleheaded mixture of conflicting views, sometimes called impartiality, and often no better than no opinion at all."

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Introduction

Otto Heinrich Warburg: 1931 Nobel laureate in physiology or medicine

Otto H. Warburg (October 8, 1883–August 1, 1970) was born in Freiburg, Baden (Germany). His father, the physicist Emil Gabriel Warburg (1846–1931), was a good friend of Albert Einstein (1879–1955) and had experimentally verified Einstein's one-photon—one-electron transfer concept in photochemistry (law of photochemical equivalency). Warburg was trained in chemistry in Freiburg and Berlin. Working with Nobel laureate Emil Fischer (1852–1919), he obtained his doctorate in 1906. He then worked to get a

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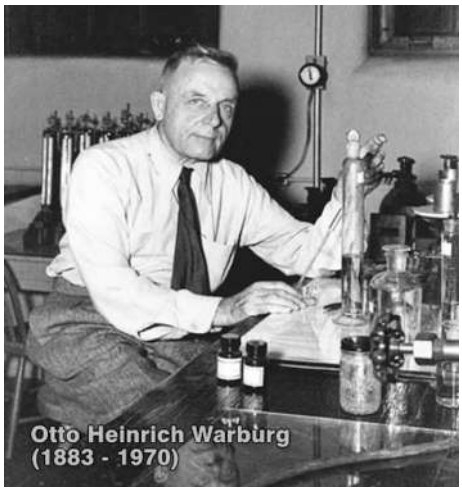

Fig. 1 A 1948 photograph of Otto Warburg and the typical equipment he used. Source: Archives of the University of Illinois at Urbana-Champaign, as published on the cover of Science (volume 108 (Issue # 2811), November, 12, 1948); also see the cover of the book by Apple (2021)

doctorate in medicine in 1911 from Heidelberg. From 1913 onward, he pursued research as a member of the Kaiser-Wilhelm Gesellschaft in Berlin-Dahlem, from 1930 to 1967 as the director of the institute of cell physiology (since 1953, Max-Planck-Institut für Zellphysiologie). Otto Warburg worked on an enormous number of topics in physiology, biochemistry, biophysics, and medicine, the latter with focus on cancer research (tumor metabolism). His research ranged from: chemistry of polypeptides; process of oxidation in chemistry; biochemistry of respiratory enzymes in mitochondria; mechanism of oxygenic photosynthesis; requirement of flavins and nicotinamide as active groups in hydrogen transferring enzymes. In 1931, he received the Nobel Prize "for his discovery of the nature and mode of action of the respiratory enzymes". (See < https://www.nobelprize.org/prizes/medicine/1931/warburg/biographical/ >) Further, he has shown that cancerous cells can live and develop, even in the absence of oxygen (see e.g., Apple 2021).

Figure 1 shows a photograph of Otto Warburg, working in Robert Emerson's laboratory at the University of Illinois at Urbana-Champaign (UIUC); here, he is wearing a tie, as was usual in those days (also see the cover of a book by Apple 2021); it reflects who he was and what he did.

On the personal side, Otto Warburg remained unmarried and shared a villa with Jacob Heiss, his secretary and friend, for more than 50 years. Having been a cavalry officer in World War I, Warburg's main sport was horse riding, which he did regularly for one hour before starting his workday at 8 AM. Despite his Jewish family background, Warburg continued his scientific work throughout World War II as an institute director with his team of technical assistants. Further interesting details of Warburg's remarkable curriculum vitae can be found in the biographical article by Hans Krebs (1972), who was one of three Nobel laureates trained as postdoctoral fellows in Warburg's Berlin laboratory.

Otto Warburg and photosynthesis

We do not describe here Warburg's extensive research but point out that his philosophy was expressed in the quote given at the top of this "Letter", advocating the truth that comes out of clearly stated errors. And indeed, in his research on photosynthetic oxygen evolution, Warburg held on to two ideas that turned out to be erroneous: (1) evolution of one oxygen molecule, in photosynthesis, requires 3–4 quanta of light, whereas we now know that it requires a minimum of 8–10 quanta (see e.g., Govindjee 1999; Nickelsen and Govindjee 2011); (2) carbon dioxide (CO_2), instead of water, is the source of oxygen (recently reviewed by Shevela et al. 2012).

Warburg elaborated on his second erroneous idea in the 1950s when he proposed that light-driven CO_2 reduction leads directly to O_2 release (see e.g., Warburg and Krippahl, 1958). In earlier experiments, however, Warburg and his technical assistant Wilhelm Lüttgens had provided evidence for O_2 formation by light-driven water oxidation to be coupled to the stoichiometric reduction of added quinone, and thus uncoupled from CO_2 reduction. These earlier experiments by Warburg on the light-driven water-quinone oxidoreductase activity of the (broken) chloroplast—a functionality now assigned to photosystem II—are the subject of three articles published in German and Russian during World War II and the first post-war year. We have now translated these historical articles and briefly comment on their content. As in most of Otto Warburg's investigations, the central biophysical method was the precise quantification of gas formation and uptake (mostly O_2) by means of a "Warburg manometer". A comprehensive description of manometric gas detection methods is provided by Lighton (2008), which may clarify the literally translated terms in the description of the glass instruments used by Warburg and Lüttgens.

Highlights of two brief communications, published in German, by Warburg and Lüttgens in 1944

Warburg and Lüttgens (1944a, see "Appendix 1") illuminated chloroplasts and reported, in April 1944, evidence for qualitative and stoichiometric validity of the following balanced equation:

$$2\,\text{quinone} + 2\,H_2O \Rightarrow 2\,\text{hydroquinone} + O_2 \quad (1)$$

The quinone used was not the native plant plastoquinone, but instead a benzoquinone added as an artificial electron

acceptor. The chloroplasts were collected from crushed spinach leaves by a single centrifugation step. In their study published in October 1944 (Warburg and Lüttgens 1944b; "Appendix 2"), a differential centrifugation protocol was used resulting in "granules". Most likely, both protocols resulted in breakage of chloroplast envelope membranes and accumulation of thylakoid membranes in the centrifugation sediment. In both preparations, the CO_2 reduction could no longer proceed. In 1944, Warburg and Lüttgens were the first to report chlorophyll-dependent, light-driven oxygen evolution coupled to quinone reduction, which may be now viewed as the remarkable discovery of the photo-enzymatic activity of photosystem II (PSII). However, the benzoquinone reduction might also have occurred at the acceptor side of photosystem I (cf. below). Warburg published his 1944 papers without any citations; he did not refer to the work Robert (Robin) Hill previously had published in England in 1937 and 1939. Robin Hill used spectral changes accompanying oxygen binding to hemoglobin to detect and quantify the oxygen evolved from a reaction that was coupled to the stoichiometric reduction of ferric oxalate (Hill 1939; preliminary results in Hill 1937). In light of the complexity of aqueous iron chemistry, the meaning of Hill's results is not fully unambiguous, but likely Hill also had observed the light-driven evolution of O_2 molecules stemming from water oxidation coupled to a reduction reaction (of Fe^{3+}) at the (electron) acceptor side of PSII.

Soon thereafter, Warburg and Lüttgens (1944b) continued these measurements with broken chloroplasts (what they called "granules") not only from spinach, but also from sugar beet leaves. Here, two discoveries were made (see "Appendix 2"): (1) role of chloride for oxygen evolution; and (2) inhibition of light-driven oxygen evolution by o-phenanthroline, for which Warburg stated "[it] proves that a dissociating heavy-metal compound is involved". We may now speculate that this could have been what we now know as the "non-heme" iron on electron acceptor side of PSII or perhaps ions from the water-oxidizing manganese-calcium cluster of PSII (see e.g., Shevela et al. 2021).

Highlights of a detailed article, published in Russian, by Warburg and Lüttgens (1946)

In this paper, concepts discovered in 1944 were expanded in a detailed manner, although the two brief 1944 communications were not cited. See "Supplementary Material" for the complete English translation of Warburg and Lüttgens (1946) paper. Less than a year after the war between the Soviet Union and Germany, this article by Warburg was published in the Soviet journal Biokhimiya (Biochemistry), underscoring Warburg's international recognition. The editor-in-chief of the journal was A.N. Bach (1857–1946), a highly influential biochemist in the Soviet Union, who had lived in France and Switzerland from 1890 to 1917, working on photosynthetic carbon assimilation and respiratory enzymes. The 1944/1946 articles of Warburg indeed confirmed a hypothesis Bach had presented already in 1893, which Popov and Zvyagil'skaya (2007) have summarized as follows: "Bach succeeded in giving a new explanation to the essence of sugar formation, considering the carbon assimilation as a redox reaction proceeding at the expense of water elements. Based on this postulate, he assumed that water rather than carbonic acid, as it was believed, was the source of evolving molecular oxygen". However, see a question on this topic in the Supplementary Material, section A.

In Warburg's 1946 article, the papers of Robin Hill (1939) on the use of hemoglobin, as well as that of Wilhelm Menke (1938a, 1938b) on the composition of chloroplasts were recognized. The paper of Warburg and Lüttgens (1946) provided (i) details on the composition of chloroplasts in terms of proteins, lipids, chlorophyll, phosphorus, iron, manganese and zinc; (ii) the possible involvement of iron (via extension of measurements on o-phenanthroline, involving reversibility of the o-phenanthroline effect); (iii) the involvement of chloride in oxygen evolution; (iv) a remark on the inhibition of the above -mentioned reactions under high light; and, interestingly, (v) the observation that quinone acts as an electron acceptor even in whole cells of a green alga (Chlorella). One could have taken the last observation to imply that, by using benzoquinone as an electron acceptor, Warburg had "chemically isolated" the light-driven reactions of photosystem II, namely water oxidation *directly* coupled to plastoquinone reduction. However, in his experiments the quinone reduction may also have occurred at a later stage of the electron transfer chain, involving the light reactions of both photosystems of oxygenic photosynthesis, PSII and PSI. We know from other data in the literature that some added electron acceptors, including benzoquinones, are reduced also by electrons from PSI, evidenced inter alia by the Emerson Enhancement Effect (R. Govindjee et al. 1960). We note that in 1944/1946, there could not have been any thought on photosystems I and II by Warburg.

Concluding Remarks

The diagram (Fig. 2) below provides a summary of the take-home messages of the three 1940s papers of Otto Warburg—giving us a glimpse of some of his outstanding discoveries during the last part of the World War II (1939–1945) under presumably difficult conditions.

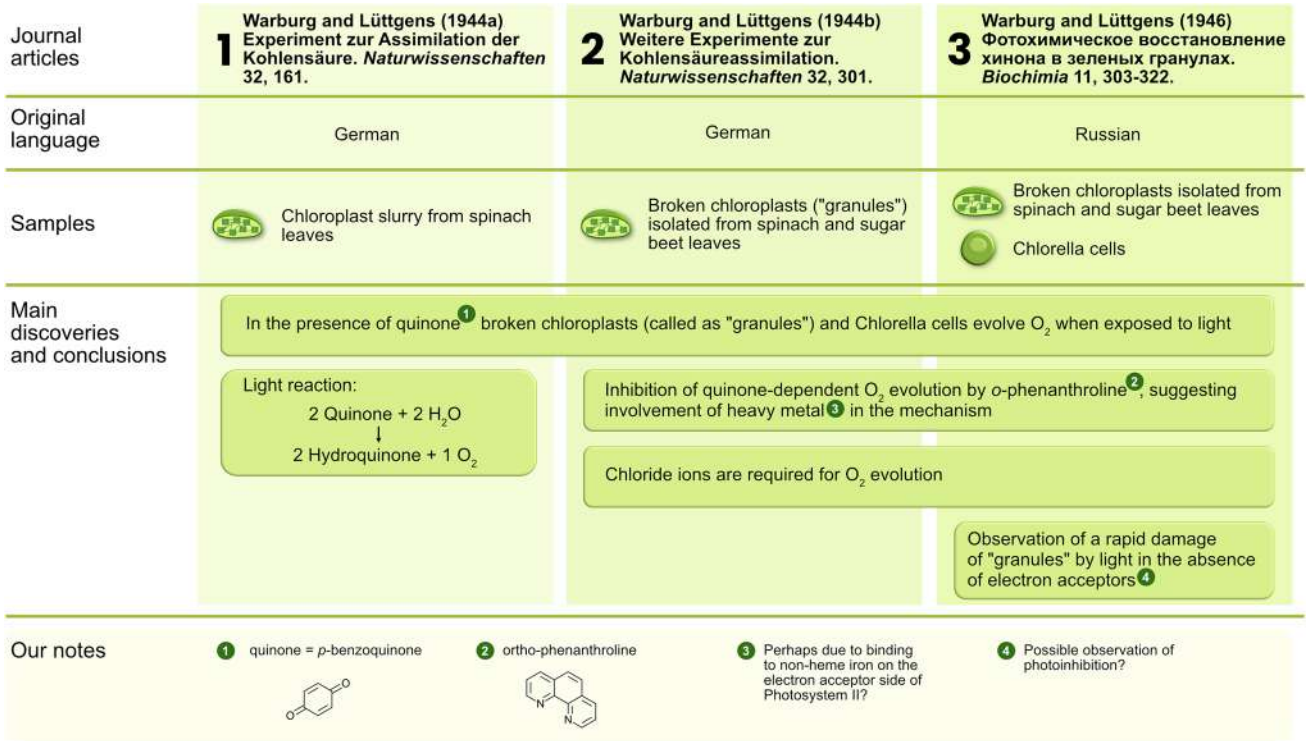

Fig. 2 Highlights of the three papers of Warburg and Lüttgens, published in the 1940s

Appendix 1

Warburg and Lüttgens (1944a), published in German, translated by Holger Dau

Naturwissenschaften, 32, Issue 14/26, April/June 1944, page. 161

Short original communications

Experiment on assimilation of carbonic acid

Since we started experimenting on carbon dioxide assimilation, we know that the chloroplast separated from green plant cells no longer is able to photochemically reduce carbonic acid. We have found a photochemical reaction that isolated chloroplasts can drive and during which molecular oxygen is split off, as is the case in carbonic acid assimilation, namely the reduction of quinone according to the equation:

$$2\,\text{quinone} + 2\,H_2O = 2\,\text{hydroquinone} + O_2$$

This equation is merely a balance equation, not meant to express the mechanism of the reaction.

Experiment

Crushed spinach leaves are pressed through a cloth; the chloroplast substance obtained by centrifugation of the spinach juice is resuspended in M/20 phosphate buffer of pH 6.3. 2 cm^3 of the green suspension, which contains about 7 mg [of] chlorophyll, are given into the main chamber of a conical manometer flask. The center well remains empty; the bulb contains 2 mg para-benzoquinone; the gas space is filled with argon.

If you add the quinone into the main chamber, nothing happens in the dark; in particular, there is no carbonic acid formation. Illumination with light of the visible spectral range initiates the evolution of oxygen, which under our experimental conditions was completed within about 1 h. 170 cm^3 of oxygen—that is about 80% of the oxygen amount predicted by the above equation—emerged when using 2 mg quinone. Without the addition of quinone, oxygen did not evolve upon illumination.

During illumination, the amount of chlorophyll remained constant. The chlorophyll absorbs the light and thereby provides the energy needed for the reaction. Whether it reacts chemically with the quinone and recovers, we cannot say. At least, chlorophyll is not involved in the overall reaction balance. In the described example, the mol oxygen/mol chlorophyll was around 10:1.

Kaiser Wilhelm Institute for Cell Physiology, the 11th of February, 1944.

OTTO WARBURG. WILHELM LÜTTGENS.

Appendix 2

Warburg and Lüttgens (1944b), published in German, translated by Holger Dau

Naturwissenschaften, Volume 32, Issue 40/43, October 1944, page. 301

Short original communications.

Further experiments on carbon dioxide assimilation

Recently, we reported in this journal[1] that upon illumination, green plant cells and the chloroplasts separated from these cells reduce quinone coupled to detachment of molecular oxygen. The balanced equation for the reaction is:

$$2\,\text{quinones} + 2\,H_2O = 2\,\text{hydroquinones} + 1\,O_2 — 52\,000\,\text{cal.}$$

The light absorbed by chlorophyll supplies the necessary energy. The photochemical yield is equal in red, yellow and blue light if one subtracts in the blue, the light absorbed by the yellow dye substances.

We have found out by now that this photochemical reaction is not bound to the structure of the chloroplast. When chloroplasts are broken, whose diameter is about 5 µ, to yield granules of diameter 0.5 µ, the photochemical yield remains unchanged.

We get the green granules from crushed spinach or sugar beet leaves pressed through linen cloth, followed by fractionated centrifugation of the juice. First, after low-speed centrifugation (1200 times gravity acceleration), the sediment is discarded. Then for high-speed centrifugation (22,000 times gravity acceleration), the green granules sediment. They contain, calculated based on dry substance, about 9.3% chlorophyll, 0.25% phosphorus, 0.1% iron, 0.017% manganese, 0.007% zinc, and in total 3% ashes.

In experiments with the green granules, we found, among others, the following:

1. The photochemical efficacy of granules disappears when dialyzed against water, or when washed with water in a centrifuge. Chloride, bromide, iodide and nitrate ions—but not rhodanide, sulfate or phosphate ions—restore the photochemical activity. Especially effective are chloride ions. 1/30000 N KCl reactivated detectably, 1/3000 N KCl reactivated the granules almost completely.
2. The photochemical efficacy of the granules also disappears when adding o-phenanthroline. *n*/10000 phenanthroline inactivates the granules completely.

Upon removal of the phenanthroline, the efficacy returns. This specific and reversible inhibition by a heavy-metal complexing agent proves that a dissociating heavy-metal compound is involved in the photochemical reduction of the quinone in the green granules.

Experimental set-up

The main chamber of a manometer vessel contained the green granules in 2 cm^3 of M/20 phosphate at pH 6.3 to 6.5. The amount of chlorophyll in the granules was 1 micromole. The gas space contained argon, the reaction vessel contained 2 mg = 18.5 micromoles of quinone. After adding the quinone, the main chamber was illuminated with a metal filament lamp such that oxygen evolution (180 cm^3 = 87% of theory) was completed in 30 to 60 min.

Berlin, Kaiser Wilhelm Institute for Cell Physiology, the 14th of October, 1944.

OTTO WARBURG. WILHELM LÜTTGENS.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11120-021-00858-8.

Acknowledgements One of us (Govindjee) thanks Sam Apple for providing him, in advance, a copy of his 2021 book on Warburg's contributions to Cancer research.

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Supplementary Material

for

Three overlooked photosynthesis papers of Otto Warburg (1883—1970), published in the 1940s in German and in Russian, on light-driven water oxidation coupled to benzoquinone reduction

by

Holger Dau, Boris Ivanov, Dmitry Shevela, William H. Armstrong, and Govindjee Govindjee

Prelude

This Supplementary Material includes first (A) a question regarding the opinion of Popov and Zvyagil'skaya (2007) on what Bach (1893) stated about the origin of oxygen in photosynthesis; and then (B) the English translation of the Russian paper "The photochemical reduction of quinone in green granules"

(A)Did Bach really state that oxygen comes from water, not CO_2, as implied by Popov and Zvyagil'skaya (2007)?

by

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In A.N. Bach's view (see Kretovich 1983), the effect of light results in the decomposition of bicarbonate (H_2CO_3), with the formation of per-carbonic acid ($H_2\ CO_4$) and formaldehyde (HCHO), as shown below:

$$3\ H_2CO_3 \text{ ---> } 2\ H_2\ CO_4 + HCHO \quad \text{(Eqn. 1)}$$

Percarbonic acid then decomposes to give:

$$2H_2CO_4 \text{ -> } 2CO_2 + 2H_2O_2 \quad \text{(Eqn 2a)}$$

$$2H_2O_2 \text{ -> } 2H_2O + O_2 \quad \text{(Eqn 2b)}$$

Hence, Bach assumed that carbon dioxide assimilation by green plants is based on a conjugated redox reaction that involves water (see Bach 1893).

Although the above is based on the earlier Baeyer's formaldehyde hypothesis (see Graßhoff 2007), it is, however, not how the process takes place (Blankenship 2021; Shevela et al. 2019). Bach's idea that photosynthesis represents a conjugated redox process is certainly correct. However, in no way Bach suggested that oxygen comes from water, as was implied by Popov and Zvyagil'skaya (2007) when they wrote: "Based on this postulate, he [Bach] assumed that water rather than carbonic acid, as it was believed, was the source of evolving molecular oxygen", citing Bach (1893).

Bach's (1893) paper has, however, the following statements on this question:

Under " Discussion of analogous reaction", he wrote :

" We only know of one class of compounds, which at ordinary temperatures, decompose to form oxygen, peroxides. So that carbonic acid can decompose in the green parts of plants with the release of oxygen, an unstable peroxide must be either directly or indirectly formed. "

And then under "Conclusions", he wrote :

"During assimilation, carbonic acid reacts to become its hydrate, CO_3H_2. In order for this reaction to double with light and the release of oxygen, an unstable peroxide intermediate must be formed." …

Then, he added:

"These results correspond exactly to the hypothesis of of M. Bayer: of the 3 molecules of carbonic acid involved in the reaction only one splits into oxygen and formaldehyde" and "The splitting of carbonic acid can only take place in the presence of substances that stabilize the active oxygen or formaldehyde".

In **conclusion**, we seriously question the statement ascribed by Popov and Zvyagil'skaya (2007) to Bach (1893) about oxygen coming from water. However, for a statement by M. de Fourcroy (1787), and later, in 1930, by René Bernard Wurmser, see Joliot et al. (2016). If anyone has a different opinion, Govindjee would appreciate receiving an e-mail.

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(B) English translation of the Russian paper "The photochemical reduction of quinone in green granules"

The following is the English translation of O. Warburg and W. Lüttgens's 1946 paper in Russian (Фотохимическое восстановление хинона в зеленых гранулах), published in Biokhimiya, volume 11(4), pages 303-322, **by Boris Ivanov (ivboni@rambler.ru).**

The original Russian paper was submitted on February 26, 1946. The figures, in the translated paper were redrawn **by Dmitry (Dima) Shevela (dmtriy.shevela@umu.edu);** and read **by Govindjee (gov@illinois.edu)** for language who asked questions, which were then answered by Boris Ivanov and the current text has been modified accordingly. Some additional minor text within square brackets has been added by Govindjee and Ivanov. At the end, we have added an Appendix (by Govindjee); it has complete references cited in Warburg and W. Lüttgens (1946).

In addition, we refer the readers to another earlier and independent translation of the same paper by **A. Lawson (1949),** which was published as a chapter "The photochemical reduction of quinone in green cells and granules" in a book by Otto Warburg titled "Heavy Metal Prosthetic Groups and Enzyme Action", published at the Clarendon Press, Oxford University Press, UK.

The photochemical reduction of quinone in green granules[#,*]

Otto Warburg, Wilhelm Lüttgens

Kaiser Wilhelm Institute of Cell Physiology, Liebenberg

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Figures redrawn by

Dmitry Shevela (Dmitry.shevela@umu.se)

[*Introduction*]

By crushing dried green leaves in water the obtained material produces oxygen upon illumination. Mölisch [1] was the first to show this effect in 1925 using the luminous bacteria method. Subsequently, this fact was repeatedly verified and finally confirmed. As the observed oxygen generation was not accompanied by carbon dioxide consumption, oxygen was expected to be produced from some other source.

In 1939, Robert [*Robin*] Hill [2] designed a novel quantitative method to "replace" [*improve*] the experiments done earlier with luminous bacteria. He added haemoglobin to the mass of ground leaves. Illumination was accompanied with light-induced oxygen production and conversion of haemoglobin into oxy-haemoglobin that was optically quantified. Using this method, Hill found that upon illumination the isolated chloroplasts reduced certain ferric salts (especially ferrioxalate) to ferrous salts and produced oxygen. Ferrioxalate is known to convert to ferrooxalate and carbon dioxide in an aqueous solution in the light. Hill "managed" to rule out

this reaction and the subsequent photochemical reduction of carbon dioxide as a possible mechanism of this effect.

The experiments described here can be considered as "expanding" the work of Mölisch and Hill. This study aimed to identify and isolate a photochemically active system from green [*algal*] cells. Gas exchange measurements were performed by a-gasometric method. Upon shaking, in the presence of air, a suspension of live chlorella cells in a respirometer that contained potassium hydroxide in its inner compartment, a manometer displayed negative values of pressure caused by a cell respiration. In the light, manometer readings decreased due to carbon dioxide assimilation and reached zero if there was sufficient illumination intensity. This method is not accurate enough to determine the photochemical yield. However, it is a simple and sensitive way to detect carbon dioxide assimilation.

If in the same manner, if we shake the juices, obtained by crushing spinach or sugar beet leaves that were capable of respiration, negative pressure did not change upon illumination; thus, in squeezed juices, light does not affect carbon dioxide consumption. This remarkable event remained under all conditions.

Oxygen consumption in squeezed juices often rapidly decreased during an experiment making it difficult to compare the values obtained at different stages, *i.e.* in the dark and in the light. We tried to stabilize the process over time supplementing the experimental samples with various compounds such as pyrocatechin and hydroquinone which are oxidized to ortho- and paraquinone in squeezed juices. This provided stable oxygen consumption over time that turned out to be light-dependent and it decreased upon illumination. This led to the discovery that the "green material" from squeezed juices reduced quinone and produced oxygen in the light:

$$\mathbf{2\ quinone + 2H_2O = 2\ hydroquinone + 1O_2}$$

[This was the discovery of benzoquinone reduction by material from cells of spinach/sugar beet leaves; experiments with algal cells of Chlorella are at the end of this paper]

Naphthoquinones such as beta-naphthoquinone sulfonic acid were reduced while producing oxygen as well. Naphthoquinones in green cells such as 2-methyl-3-phythyl-1,4-naphthoquinone have not been studied yet.

Content [*sections of the paper*]

1. Oxygen consumption by green leaf extracts.

The leaves of spinach or sugar beet were put through a chopper, which provided a mass of material, which was squeezed through a tissue. This squeezed juice was briefly centrifuged (at

1200xg) to remove intact cells and insoluble salts [*the protocol in details is presented in section #3]*. Centrifugate, further referred to as to as a green extract, was a dark green liquid and consisted of a suspension of chloroplasts. Some of them were intact and some were broken down into "granules' *[mostly "broken chloroplasts"]* had an approximate pH of 6.5.

Upon shaking the green extracts in the air in a respirometer, oxygen consumption significantly declined over time. Carbon oxide at 80% (v/v) decreased this process by 50% in a light-independent manner. Therefore, oxygen transfer in the extract was mediated by copper of phenoloxidase [3]. This is consistent with the fact that oxygen consumption increased when the sample was supplemented with pyrocathechin. The elevated oxygen consumption caused by the presence of pyrocathechin gradually decreased as well. To prevent this decrease, we added an excessive amount of hydroquinone along with pyrocathechin. Hydroquinone itself is not oxidized by phenoloxidase, and hence cannot be responsible for elevated oxygen consumption by the extract. However, adding hydroquinone together with pyrocathechin induces the following reactions:

$$\mathbf{2\ Cu^{2+} + pyrocathechin = 2\ Cu^{+} + orthoquinone + 2H^{+}}$$

$$\mathbf{Orthoquinone + hydroquinone = pyrocathechin + paraquinone}$$

In this case, pyrocathechin acts as a catalyst, while the final product of the oxidation is the less toxic paraquinone produced instead of the toxic orthoquinone and the products of its further oxidation.

In fact, upon adding a small amount of pyrocathechin and an excess of hydroquinone to the green extract, the oxygen consumption was stable over time until all the hydroquinone was oxidized into the quinone.

During the experiment (see Fig.1), we determined the oxygen consumption and production of carbon dioxide in each of three samples:

No supplements	CO_2/O_2 = 0.7-1.0
+ pyrocathechine	CO_2/O_2 =0.3-0.4
+pyrocathechine + hydroquinone	CO_2/O_2 = 0

The above data implied that the oxygen consumed or transferred by pyrocathechin did not contribute to the production of carbon dioxide. At the same time, hydroquinone even attenuated the generation of carbon dioxide which had been released before it was added, and thus hydroquinone most likely replaced the native respiration substrates.

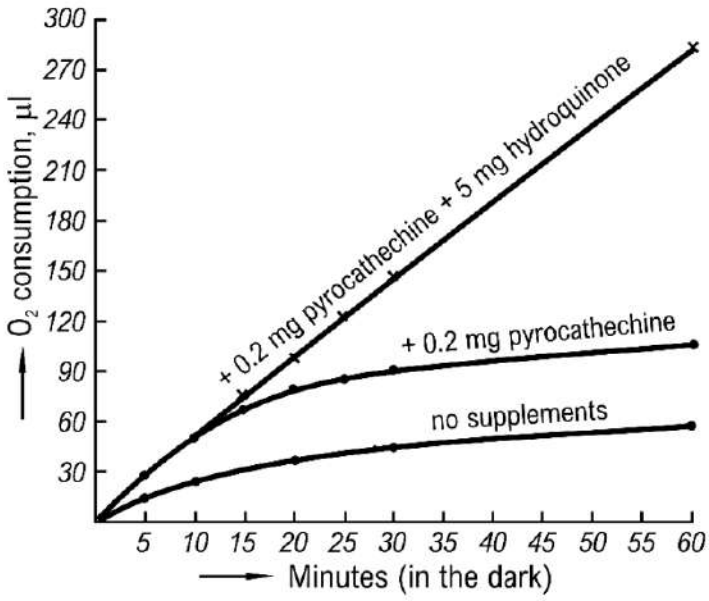

Fig. 1. Oxygen consumption by the green leaf extract in the dark. Each sample contained 2 ml of extract; 20°C, pH 6.5. The gas space contains air, the inner compartment contains KOH.

Both formed elements *[granules]* and "interstitial" fluid *[simply the fluid excluding 'granules']* of the green extract [that] onsume oxygen. If they are separated by centrifugation at pH 6.5 (pH of the extract) and supplemented with pyrocathechine and hydroquinone, 25% of initial consumption of oxygen was by the formed elements while 75% was by the interstitial fluid. If the centrifugation is performed at a more acidic pH, such as pH 5, most phenoloxidase resides in the pellet. Hence the pellet contains both phenoloxidase from the formed elements and phenoloxidase precipitated by acid from the interstitial fluid. This indicates that the green extracts should be centrifuged at pH 6.5 if polyphenoloxidase from the formed elements should be separated from polyphenoloxidase from the fluid (for instance, for photochemical tests).

2. Influence of light on oxygen consumption by green leaf extracts.

To test whether oxygen consumption by green leaf extracts is sensitive to light, two identical samples were shaken with the air. One sample was shaken in a respirometer in the dark and the other one in the light, or we used only one respirometer that was alternately placed either in the dark or in the light. The second experimental protocol is preferable if the oxygen consumption rate is stable over time.

A low voltage lamp with a metal wire (11 V, 38 A) served as a source of light. A condenser transformed it into a parallel light rays beam, which was filtered through a 2 cm of 20% acidic ferrosulfate solution, and entered a thermostat in a horizontal direction; there, it was reflected, using mirror in a vertical direction to the manometric vessels.

The samples were shaken in the air at 20°C and pH 6.5, and we obtained the following results:

The tested object	**Supplements**	**CO_2/O_2 in the dark**	**The influence of light**
Insterstitial fluid from the extract [*i.e., the fluid fraction after centrifugation*]	**No supplements**	~0.8	**No influence**
Insterstitial fluid from the extract	**Pyrocathechin**	~0.4	**No influence**
Insterstitial fluid from the extract	**Pyrocathechin Hydroquinone**	~0	**No influence**
The formed "elements" from the extract [*i.e., the granules*]	**No supplements**	~0.8	**No influence**
The formed "elements" from the extract	**Pyrocathechin**	~0.4	**Consumption O_2 inhibition**
The formed "elements" from the extract	**Pyrocathechin Hydroquinone**	~0	**The O_2 consumption ceased or O_2 was evolved**

Thus, we found that light decreases the oxygen consumption, but this influence was observed only for the "formed elements" only when they were supplemented with pyrocathechine or pyrocathechine plus hydroquinone. The results of these experiments are presented in Figs 2 and 3. As upon supplementation with pyrocathechine with hydroquinone (see Fig.3) oxygen consumption is stable over time, this experimental protocol is preferable. Yet from a theoretical point of view, the fact that the light affects oxygen consumption upon supplementation with pyrocathechine alone (Fig. 2) seems to be important as such.

From a methodological point of view, it is essential to use the freshly prepared green suspensions from the leaves that have pH 6.2-6.5. To our surprise, we did not manage to detect the effect of light at pH 7.4 though oxygen consumption during hydroquinone oxidation in the dark at pH 7.4 was the same as at pH 6.4. Furthermore, we did not observe the effect of light after 24 h incubation of the suspension on ice; at the same time, the shelf life of the suspension did not affect oxygen consumption during hydroquinone oxidation in the dark. It follows that hydroquinone oxidation in the dark and suppression of the oxidation in the light have a varying sensitivity and a varying response to certain stimuli.

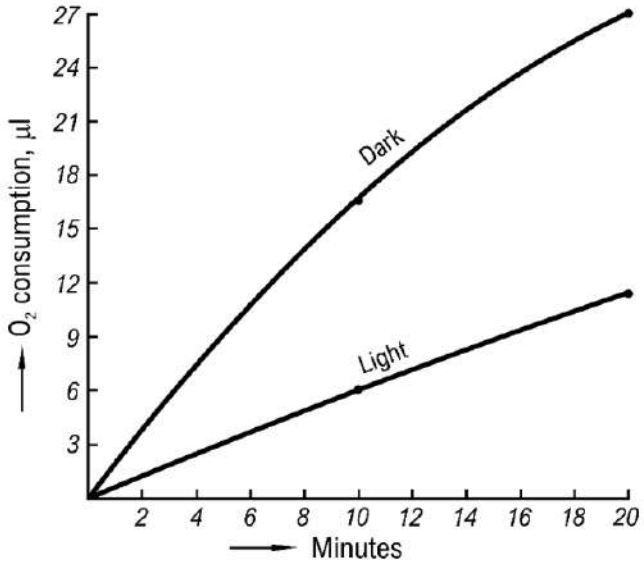

Fig 2. Light affects pyrocathechin oxidation in green formed elements *[the granules]* 0.2 mg of pyrocathechin + 2 ml of suspension. 20°C, pH 6.5. The gas space contains air.

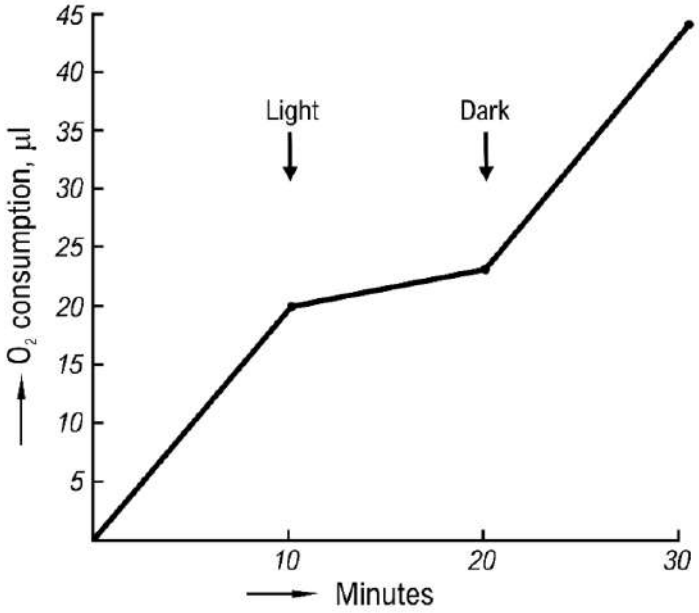

Fig. 3. The effect of light on hydroquinone oxidation in green formed elements *[the granules])*. 0.2 mg of pyrocathechin + 5.0 mg of hydroquinone + 2 ml of suspension. 20°C, pH 6.5. The gas space contains air.

As chloride was further shown to be a co-ferment of the light-sensitive system *[see section #6]*, we added 0.05% potassium chloride (KCl) to the green suspension during the following experiments. In the experiments shown in Figs.2 and 3, green pellets were not washed with water

and the residual interstitial fluid enriched with chlorides was sufficient for a light-sensitive effect.

[The above shows the chloride effect]

As shown in Figs. 2 and 3, oxidation of pyrocathechine and hydroquinone was attenuated in the light which can be explained most simply if we assume in the light the possibility of a reversal of the reaction occurring in the dark; it means that in the light the reduction of quinones in the "formed elements" is accompanied by oxygen evolution.

[Fundamental conclusion]

2 orthoquinone + $2H_2O$ = 2 pyrocathechine + $1O_2$
2 paraquinone + $2H_2O$ = 2 hydroquinone + $1O_2$

Further experiments supported this interpretation.

3. The suspension of granules.

Green formed elements used for the experiments, described above, consisted of chloroplasts, intact and "degraded" to granules. A morphologically homogeneous mass can be obtained by grinding green pellets with a glass bead tightly adhering to a tube. Under these conditions, all chloroplasts were broken down into "granules" with a size of 1/10 of chloroplast diameter, yet the photochemical activity of the material was not altered.

We thrice washed the granules with water to separate them completely from the interstitial fluid, using centrifugation (at 22,000 x g) under cooling conditions, and each time we ground the pellets with a glass bead and brought the volume up to the initial volume of the "squeezed" juice.

The leaves of spinach harvested in May or at the beginning of summer and the leaves of the sugar beet harvested from the end of July to the first frost served as a source of granule suspensions. There was no difference between the freshly harvested leaves of beet and the leaves stored at 5°C for several days.

Here is a detailed description of the protocol for "granule" suspension preparation. The leaves of spinach or sugar beet were put through a chopper → the ground mass was squeezed through a tissue → the filtrate was centrifuged at 1,200xg for 5 min, the pellet was discarded → the supernatant was centrifuged at 22,000x g for 10 min → the pellet was ground with a glass bead and diluted in water up to a volume equal to an initial volume of the squeezed juice → the pellet was washed thrice by centrifuging under cooling conditions → finally, the pellet was diluted in water up to 1/3 of an initial volume of the squeezed juice and the concentration of chlorophyll in this suspension was assessed in a small sample → the suspension was diluted with water to the chlorophyll content of 0.9 mg in 1 ml.

To assess the chlorophyll concentration, aliquots of suspensions were centrifuged at 22,000x g**,** and the pellet was ground with methanol and centrifuged once more. We measured the absorbance of a centrifuged clear methanol solution at a wavelength of 578 nm and, using the absorption coefficient:

$$c = 17[\frac{Volume(cm^3)}{chlorophyllmass(mg)}]$$

The chlorophyll concentration was calculated as:

$$\beta = \frac{ln\frac{i_0}{i}}{17d}[\frac{chlorophyllmass(mg)}{Volume(cm^3 \mid \quad)}]$$

The activity of the granules gradually declined with storage on ice. To stabilize it, we added 1/10 volume of m/2 phosphate and 1/10 volume of 0.5% KCl. Under these conditions, activity decreased by 15% in 24 h at +5°C.

To obtain the material to conduct experiments in winter, we prepared dry samples. Drying the frozen granule suspension under vacuum led to a 50% decrease in photochemical activity. Storing the dry powder caused further gradual decline of its activity. We did not obtain any satisfying results using the leaves that were frozen and dried under vacuum. Thus, we had to use the freshly prepared material for our experiments.

The chemical composition of chloroplasts that served as a source of the granules was estimated by W. Menke [4]. He found that chloroplasts consisted of: 48% proteins, 37% lipoids including 8.6% chlorophyll, 8% "cinder" *[ash]*. We found that granules consisted of:

- 9% chlorophyll,
- 3% "cinder" *[ash]* ,
- 0.3% phosphorus (1 mol per 1 mol of chlorophyll),
- 0.1 % iron (0.18 mol per 1 mol of chlorophyll),
- 0.016% manganese (0.029 mol per 1 mol of chlorophyll),
- 0.068% zinc (0.010 mol per 1 mol of chlorophyll).

4. The photochemical reduction of quinone

Commercial chemically pure quinone was distilled with aqueous vapor, following the protocol of Vanino *[reference was not given]*. Light yellow crystals precipitated in the condenser tube were dried under vacuum. Prior to an experiment, they were dissolved into a 1% solution under cooling conditions. If water is used as a solvent, the light yellow aqueous solution immediately darkens, and the resulting solution contains a compound that irreversibly inhibits the photochemical activity. The formation of a dark-colored poisonous substance (which occurs without involving oxygen from the air) can be avoided if quinone was dissolved in n/100 sulfuric acid which was therefore further used as a solvent. Adding pure quinone to a granule suspension at pH 6.5 in the absence of oxygen did not induce any changes, and, it is important to note that no gas production was observed, or only the trace amounts of carbon dioxide had evolved. Upon illumination with a filament lamp, oxygen was evolved until the quinone was fully depleted, the

rate of oxygen evolving being proportional to the illumination intensity. As seen in Fig.4, we detected 80-90% oxygen, calculated according to the equation:

2 quinone + 2 H_2O = 2 hydroquinone + 1O_2

The reaction was attenuated or abolished at pH 7.4. The granules should not be illuminated prior to adding quinone as granules are rapidly damaged in absence of photochemical substrates in the light.

[This may be considered as one of the earliest indication(s) of photoinhibition.]

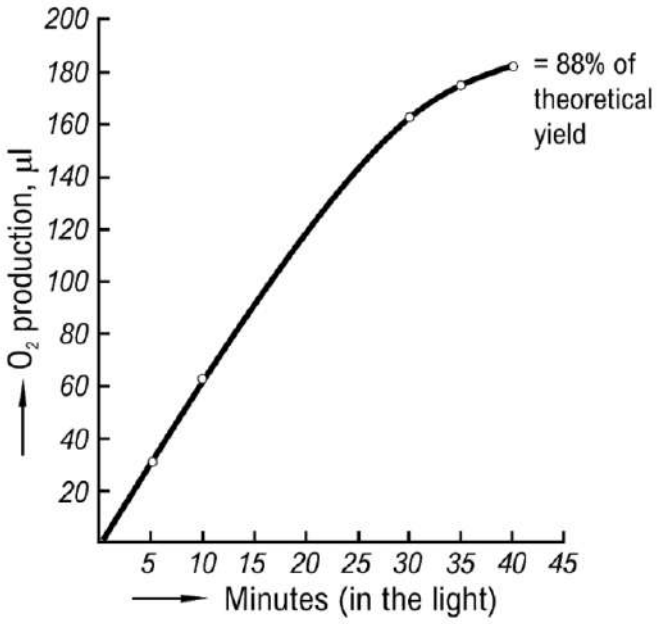

Fig. 4. The photochemical reduction of quinone in the green granules. 2 ml of the "granule" suspension, ***m***/20 phosphate, pH 6.5; 0.05% KCl; the sample contains 0.9 mg = 1 µmol of chlorophyll. 20°C. The gas space contains argon. 2 mg of quinone (18.5 µmol in 0.2 ml of ***n***/100 H_2SO_4) was added to the sample at t_o timepoint, and the sample was illuminated.

The experiment presented in Fig.4 was complemented with the following controls:

1) The granule suspension was illuminated in the same manner as in the experiment but without quinone supplementation. No gas was produced.

2) The inner reservoir contained phosphorus. Upon illumination of a respirometer, no gas was generated. From these data, we can conclude that the evolved gas was oxygen.

3) Adding 10 mg of Na_2S0_4 and **0.1** ml of $\boldsymbol{n}/10$ H_2SO_4 to 2 ml of the granule suspension and centrifuging produced a clear supernatant that could be used to assess quinone content by iodometric titration, according to Valeur [5]. If the experimental sample had been illuminated until oxygen production ceased, the entire quinone turned out to be consumed; while its amount in the sample placed in the control respirometer in the dark almost did not change.

We did not manage to perform the same experiment using ortho-benzoquinone due to its instability. However, in the light, the green granules reduced beta-naphthoquinone sulfonic acid, one of the orthoquinones, with the oxygen generation. Taking into consideration this fact and the

fact that pyrocathechine oxidation was suppressed in the light, we should assume that, similar to paraquinone, ortho-benzoquinone in green granules was reduced in the light that accompanied oxygen generation. If this assumption is true, we can expect that light will inhibit all types of respiration mediated by oxygen transfer by phenoloxidase, as, according to Raper [6], orthoquinones are the intermediates in this reaction. From the other point of view, the "respiration" of green granules is undoubtedly not sensitive to light in the absence of pyrocathechine. *[The term 'respiration' is used here as a synonym of oxygen consumption]*.

5. Regarding the chemical equation

The equation of the photochemical reduction of quinone

$$\mathbf{2\ quinone + 2\ H_2O = 2\ hydroquinone + 1O_2\ -52\ 000\ cal,}$$

reflects only the balance of this reaction and does not describe its mechanism. There is no doubt that thermolabile "ferments" are involved in this reaction as the photochemical activity of a granule suspension declines by one half after 10 minute heating at 40°C and is abolished after 10-minute heating at 50°C. At the same time, no changes in chlorophyll and no signs of coagulation or agglutination of the granules were observed.

Our chemical equation is based on the assumption that a compound in the granule was a catalyst. Any component of the granule can participate in reactions, but every change it undergoes should be reversible. Chlorophyll does not disappear during the reaction. If we assume that it reacts with quinone, then it would revert to its initial state for 92 times during the experiment II (Fig.5), as 18.5 μmol of quinone was reduced in a suspension containing 0.2 μmol of chlorophyll.

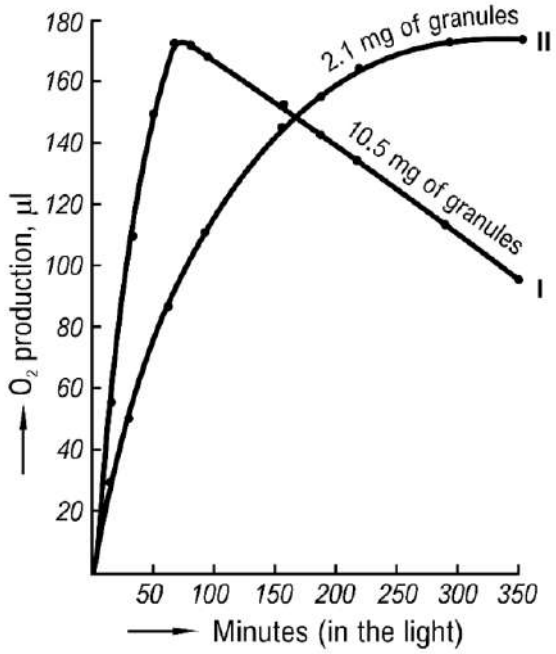

Fig. 5. Dependence of the photochemical reduction of quinone on granule content. 20° C. ***m***/20 phosphate pH 6.5; 0.05% KCl. The gas space contains argon. 2 mg = 18.5 µmol quinone in 0.2 ml , ***n***/100 H_2SO_4 was added to the samples at t_0 timepoint, and the samples were illuminated with red light (at wavelength >610 nm **I**: 2 ml of granule suspension contain 10.5 mg of dry matter including 1 µmol of chlorophyll; **II**: 2 ml of granule suspension contain 2.1 mg of dry matter including 0.2 µmol of chlorophyll.

This experiment solves a more general problem: is there any component of a granule consumed during the photochemical reaction? During experiment II (see Fig.5), the amount of converted quinone was equal to the amount of dry matter of the granules. If we assume that the partner of quinone, as well as quinone itself, had a small molecular weight (108 for quinone), the entire mass of the granule would be involved in this reaction. Thus, we can conclude that the granule content participates in the photochemical reduction of quinone not stoichiometrically but as a catalyst, which is supported by our balanced equation.

The fact that the yield of the generated oxygen is less than 100% and reaches only 80-90% at least partially results from oxygen consumption by the granules. It is substantially lower than the photochemical oxygen generation in the bright light but it should not be neglected (experiment I, Fig.5). If the gas space contains air instead of argon, the oxygen consumption increases as well as the deficiency of the photochemically generated oxygen *[i.e., the calculated amount of evolved oxygen decreased]*.

One should not think that oxygen consumption by final products of the photochemical reduction of quinone is a reverse reaction as the granules consume oxygen even in the absence of hydroquinone. At the same time hydroquinone alone without pyrocathechine cannot increase oxygen consumption by the granules. But the most important is the following observation. In a photochemical experiment, the light-dependent oxygen generation rate and the endpoint volume

of generated oxygen upon supplementation with both quinone and hydroquinone are equal to the oxygen generation rate and the endpoint volume of generated oxygen upon supplementation with quinone alone. These results are shown in Fig.6.

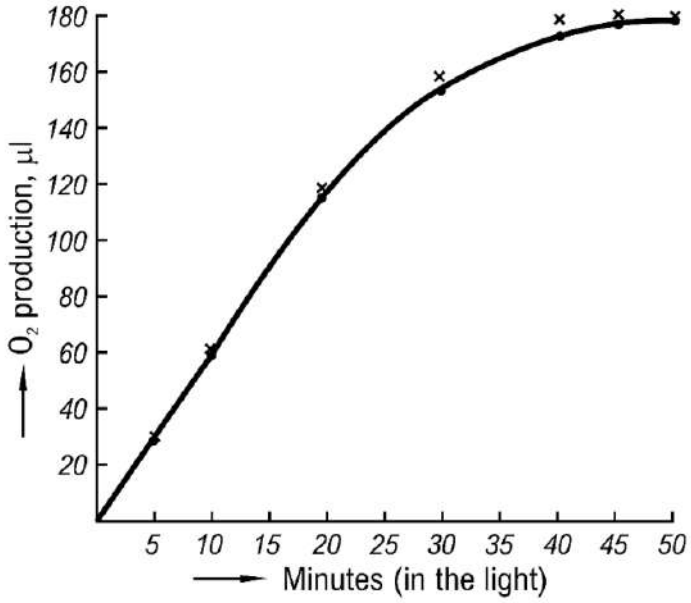

Fig. 6. The photochemical reduction of quinone upon supplementation with hydroquinone.
2 ml of granule suspension contains 1 µmol of chlorophyll. 20°C, ***m***/20 phosphate pH 6.5; 0.05% KCl. The gas space contains argon.

•: 6 mg of quinone in 0.2 ml ***n***/100 H_2SO_4 was added to the samples at t_0 timepoint, and the samples were illuminated, ×: 2 mg of quinone + 2 mg of hydroquinone in 0.2 ml ***n***/100 H_2SO_4 were added to the samples at t_0 timepoint, and the samples were illuminated.

52,000 cal required for the generation of 1 mol of oxygen according to our balanced equation are provided by light absorbed by chlorophyll. If the illumination intensity and quinone concentration are constant, the oxygen generation rate will be higher the higher will be the concentration of the granules. For a given illumination intensity, the maximal reaction rate can be reached if the concentration of granules is high enough to absorb the entire light incident on the suspension. We may consider the absorption of light by quinone negligible compared to the absorption of light by chlorophyll in our experimental design. Moreover, quinone absorbs light only in the blue range of spectrum while the light of the entire visible light spectrum absorbed by chlorophyll is active in the photochemical reduction of quinone. In the experiments shown in Fig.5, oxygen generation was achieved by illumination with red light. In conclusion, we would like to indicate that apart from quinone consumption and oxygen generation, the generation of hydroquinone should have been estimated. But this has not been done yet.

6. The co-ferment

In the protocol of suspension preparation described above, we indicated that the granules were washed by water with centrifugation. During this manipulation, the photochemical activity of the granules declined when the residual "cellular juice" was removed. After the granules were thrice washed with water and the supernatant was carefully removed, they lost much of their photochemical activity. When we added the cellular juice to a granule suspension, the photochemical activity of the granules was restored. Adding cellular juice in the amount of 1/500 of suspension volume significantly restored the activity while adding 1/10 volume restored it completely. Adding more "cellular juice" should be avoided, as it consumes quinone in the dark reaction and thus the excess juice could decrease the yield of the photochemically generated oxygen.

The photochemically active compound of cellular juice is stable during boiling and moreover it is stable upon calcination *[roasting]*. It is nothing more than chloride contained in the cellular juice at a concentration of 0.08 M. Therefore, the photochemical activity of the washed granules can be restored by adding KCl with the same efficiency as by adding cellular juice (see Fig. 7). Adding 1/5000 *N* KCl significantly restored the activity while adding 1/150 *N* KCl fully restored it.

Although chloride is a true co-ferment of the cellular juice, it could be substituted by other anions such as bromide, iodide, or nitrate. Cl^- and Br^- were equally active while I^- and NO^-_3 were significantly less active. Fluorides, rhodanides, sulfates, phosphates, and all tested cations were inactive. The fact that the light absorbed by chlorophyll becomes chemically active only in the presence of certain anions shows that theories about mechanisms of carbon dioxide assimilation are by far premature.

We showed that chloride ion was a co-ferment for photochemical reduction of oxidized iron salts discovered by Hill. We used potassium hexacyanoferrate (III) instead of ferrioxalate used by Hill. The advantage of using this salt is that its photochemical reduction by the green granules can be detected by a manometric method, similarly to the reduction of quinone. Our experimental mixtures had pH [*in the range of*] 6.1 – 6.5. Phosphate buffer should be sufficiently concentrated to prevent a possible shift in pH below 6.1 due to the following reaction:

$$\mathbf{Fe^{3+} + H = Fe^{2+} + H^+}$$

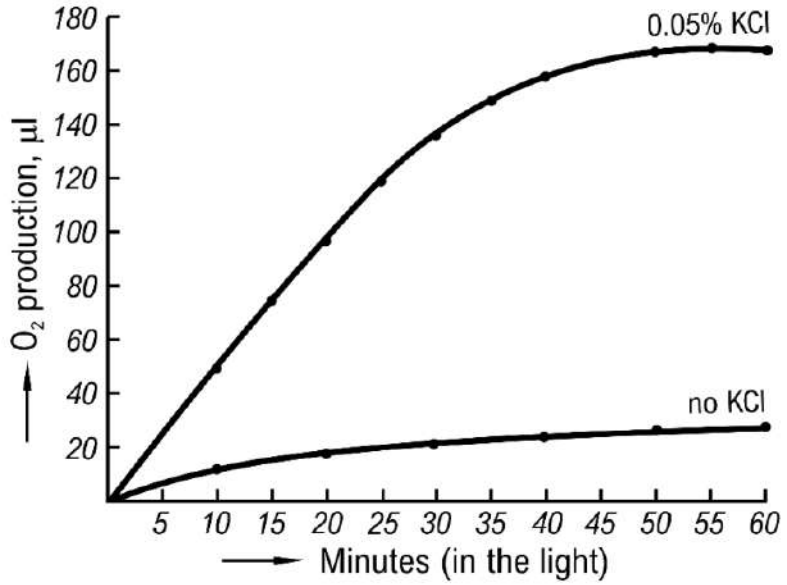

Fig. 7. Reactivation of washed granules by adding chloride. 2 ml of the granule suspensions in ***m***/20 phosphate buffer, pH 6.5, each sample contains 1 µmol of chlorophyll. 20°C. The gas space contains argon.

2 mg of quinone in 0.2 ml of ***n***/100 H_2SO_4 was added to the samples at t_o time point, and the samples were illuminated.

7. Narcotics

Octyl alcohol in a saturated solution completely abolished the photochemical activity of the granules.

Adding 0.1 mg of phenylurethane to 1 ml of a suspension suppressed the photochemical activity by 50%. Therefore, the concentration of phenylurethane in the interstitial fluid was

6.1×10^{-4} M.

This is the half-maximal inhibitory concentration of the assimilation of carbon dioxide in live chlorella cells [7].

Although both cells and chloroplasts in the scrutinized material were damaged, the surface of green granules, as in living cells, still contained compounds crucial for the photochemical reaction situated in a needed order. Soluble and insoluble ferments are not inhibited by saturated solutions of octyl alcohol or phenylurethane.

8. Orthophenanthroline

Among the compounds that specifically and reversibly react with the salts of heavy metals, cyanide and cysteine are oxidized by quinone at 20°C and pH 6.5 with carbon dioxide formation and thus cannot be used for the inhibitory tests. Carbon dioxide which is insensitive to quinone does not inhibit photochemical reduction of quinone in the green granules. In a manometric

experiment, the oxygen generation rate and the endpoint volume of generated oxygen would be the same in samples placed in two respirometers, the first one filled with argon and the second one filled with carbon [mon]oxide. Ortho-phenanthroline is one more reagent to detect heavy metal being insensitive to quinone. This reagent was synthesized by F. Blau [8] in 1898, and he observed that ortho-phenanthroline formed stable complexes with iron, nickel, cobalt, and zinc but not the manganese. 3 molecules of ortho-phenanthroline are bound to 1 heavy metal ion in each such complex.

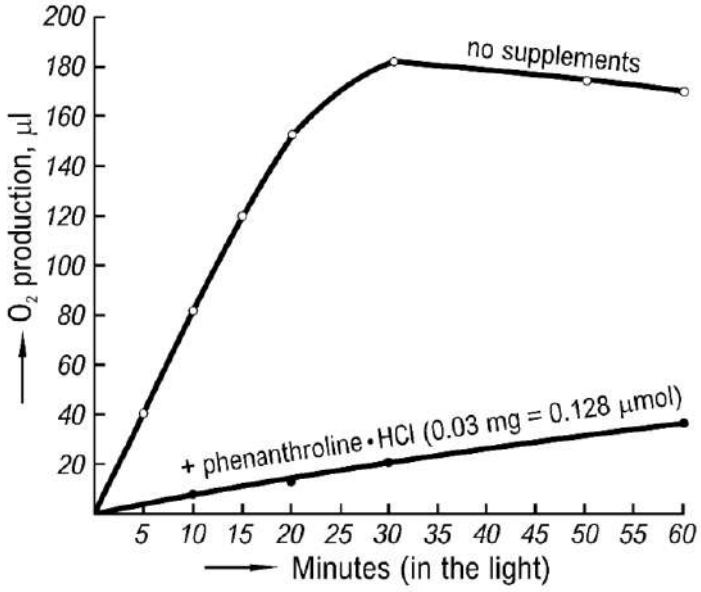

Fig. 8. Orthophenantroline inhibits the photochemical reduction of quinone.

2 ml of granule suspension in ***m****/20* phosphate buffer, pH 6.5; 0.05% KCl; each sample contains 1 µmol of chlorophyll. 20°C. The gas space contains argon. 2 mg quinone in 0.2 ml of ***n***/100 H_2S0_4 was added to a sample at t_0 timepoint.

Even at a very low concentration, phenanthroline suppressed the photochemical reduction of quinone (see Fig. 8). Adding 0.02 mg = 0. 085 µmol phenanthroline chlorohydrate (molecular weight 235) to 2 ml of granule suspension containing 1 µmol of chlorophyll inactivates the granules by one half, while adding 0.04 mg = 0.170 µmol inactivates them completely.

Therefore, the amount of phenanthroline completely inactivating the granules could bind only 1/6 of the present chlorophyll. This implies that the reaction of phenanthroline with chlorophyll could not have caused this suppression. This amount was also insufficient to bind all the iron in the granules according to the following estimation: 3 molecules of phenanthroline to 1 iron atom. And finally, this amount was in abundance to bind all the zinc in the granules. Manganese contained by the granules cannot be taken into account as it does not form stable complexes with phenanthroline.

The inhibition by phenanthroline is reversible. Adding excess zinc sulfate from the side reservoir after the measurement restored the oxygen generation rate up to the non-inhibited control. Ferrous sulfate reactivated the granules as well, however, not to the same extent as zinc sulfate (see Fig. 9). On the other hand, when we mixed the phenanthroline solution with metal salts and subsequently added the granules, ferrous iron, cobalt, and nickel along with zinc prevented the inhibition by phenanthroline. Thus, only free phenanthroline and not metal-bound phenanthroline possess inhibitory activity.

Based on our experiments, we can conclude that the inhibitory effect of phenanthroline resulted from its binding to a heavy metal crucial for the photochemical reduction of quinone. It may be thought that this metal in the granules is iron or zinc but it could not be manganese for the reasons described above. We ruled out other heavy metals such as cobalt or nickel as they were not contained by the granules.

We cannot agree with the objection that the inhibitory effect of phenanthroline had the nature of narcotic inhibition. Because if we take into consideration that some fraction of phenanthroline binds to the granules, the half-maximal inhibitory concentration is less than 4.2×10^{-5} M;while the half-maximal inhibitory concentration for a such potent narcotic as phenylurethane is 6×10^{-4} M and hence exceeds the half-maximal inhibitory concentration of phenanthroline by 15 times.

Even in very small concentrations, phenanthroline suppresses the assimilation of carbon dioxide in living chlorella cells.

Phenanthroline does not inhibit catalase activity in the granules, while similar to other catalases, this enzyme is inhibited by cyanide. We consider the absence of catalase inhibition by phenanthroline to be a very important fact as it excludes an *a priori* assumption that catalase inhibition underlies the phenanthroline-dependent attenuation.

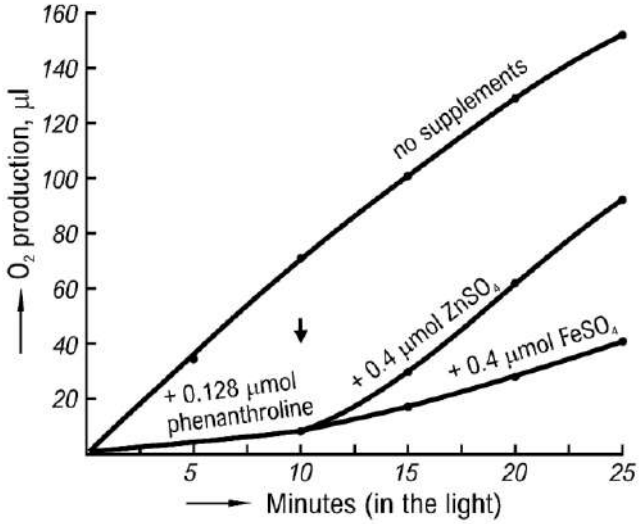

Fig. 9. Reactivation by $ZnSO_4$ or $FeSO_4$ of the granules inhibited by phenanthroline.
See Fig. 8 for the other conditions.

9. Susceptibility to acids

Adding 1/50 volume of 2M acetate buffer (pH 4) to the granule suspension containing 1 μmol of chlorophyll in 1 ml makes the pH of the mixture close to 4.2. Using the granules resuspended after centrifugation in m/20 phosphate pH 6.5 and supplemented with KCl in the experiments with quinone showed that the granules lost their photochemical activity almost entirely. Adding the centrifugate to the inactivated granules did not restore their photochemical activity.

The centrifugate contains about 2/3 zinc and only 1/50 iron present in the granules. It contains one more compound [which *absorbs at 270 nm and 340 nm, and fluoresces in the ultraviolet]*. The absorbance is so significant that it can be detected in 1 cm thick layer.

When performing the experiments, one should make sure that prior to adding the acetate buffer the granules are completely cleaned from cell juice by washing with water. The last washing buffer should not absorb in the ultraviolet.

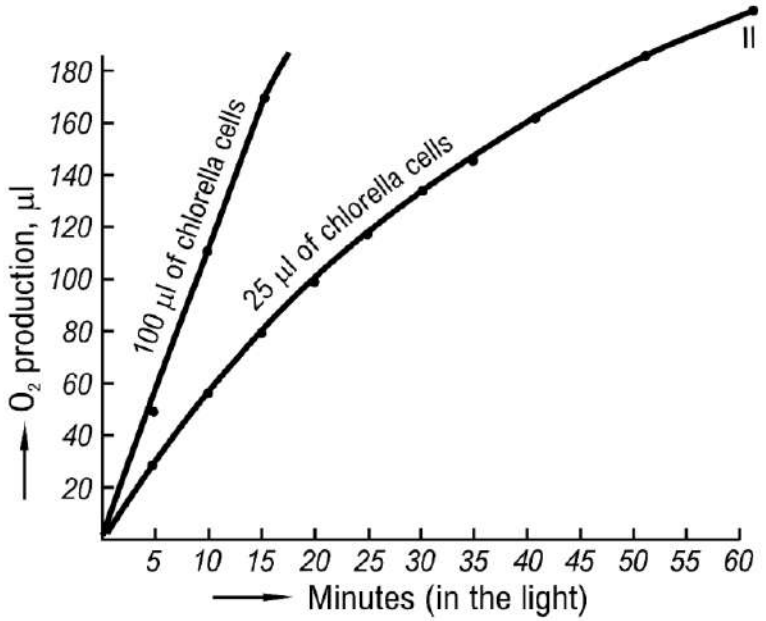

Fig. 10. The photochemical reduction of quinone by living chlorella cells. 2 ml of cell suspension containing 100 or 25 μl of cells in m/20 phosphate buffer, pH 6.5; 0.25% KCl. 20°. Argon. 2 mg = 18.5 μmol of quinone was added to the sample at t_0 timepoint, and the sample was illuminated.

10. Live Chlorella cells

Like green granules, live green algae reduce quinone in the light. As live cells are permeable to quinone, this result presented in Fig.10 could be considered obvious.

In the dark in the absence of oxygen, adding quinone to chlorella generates carbon dioxide evolving 1/50 of chlorella volume in 5 min (under conditions of a photochemical experiment). The generation of carbon dioxide was insignificant compared to the photochemical oxygen generation that reaches 1/2 or even the entire volume of chlorella suspension in 5 min. However,

it should be borne in mind to a greater extent, the lower illumination intensity is; since it can create some doubt whether the oxygen generated in the light after adding quinone was formed directly or was a secondary product originating from carbon dioxide. Such doubts do not arise from the experiments with isolated granules as the isolated granules are not capable of carbon dioxide assimilation. Thus, the isolated granules are more preferable, than live green cells to study the photochemical reduction of quinone.

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Appendix

Complete references for papers cited by Warburg and Lüttgens (1946)

With added information, *in italics,* on several scientists

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[**Note:** When there is more than one reference under one number, we have used ‘a’, ‘b’. ‘c’ and ‘d’]

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