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Historical corner

On the requirement of minimum number of four versus eight quanta of light for the evolution of one molecule of oxygen in photosynthesis: A historical note

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Abstract

A bitter controversy had existed as to the minimum number of quanta required for the evolution of one molecule of oxygen in photosynthesis: Otto Warburg had insisted since 1923 that this value was 3–4, whereas Robert Emerson and others continued to obtain a value of 8–12 since the 1940s. It is shown in this letter that the 1931 Nobel-laureate of Physiology & Medicine Otto Warburg published, in his last and final paper, just before his death in 1970, a measured minimum quantum requirement of oxygen evolution of 12 at the lowest intensities of light he used. Although using his theory on photolyte, Warburg calculated a value of 3–4 for the quantum requirement, this is the first confirmation by Warburg of the higher measured quantum requirement. However, it has remained unknown to most investigators. It is expected that this information will be of general interest not only to those interested in the history and research on photosynthesis, but to the entire scientific community, especially the writers of text books in biology, biochemistry and biophysics.

Introduction

Photosynthesis is at the heart of all life because it is the source of almost all of the food living organisms need for their life, and the oxygen used for the respiration of all aerobic organisms. The determination of the maximum quantum yield of photosynthesis (i.e., maximum number of oxygen evolved per quantum of light absorbed) is a fundamental question; it provides information on the maximum possible efficiency of energy storage in photosynthesis. The inverse of the maximum yield is the minimum quantum requirement per molecule of O_2 evolved. This number has been the subject of an intense and long-standing controversy between Otto Warburg (the 1931 Nobel-laureate of Physiology & Medicine, discoverer of several respiratory enzymes and co-enzymes, and many phenomena in modern biochemistry) and his doctoral student Professor Robert Emerson, discoverer of the *Photosynthetic Unit* (Emerson and Arnold 1932a, b), of the *red drop* in the quantum yield of oxygen evolution plotted as a function of wavelength of light (Emerson and Lewis 1943a, b), and of the *Emerson Enhancement Effect* (Emerson et al. 1957), concepts that led to the modern view of a two-light reaction and two-pigment system scheme of photosynthesis. The controversy was whether photosynthesis requires a minimum of 3–4 quanta (Warburg) of light or 8–12 quanta (Emerson) of light per oxygen molecule released. The controversy lasted for more than a quarter of century and is still described in modern texts as being unresolved in the sense that no one has devised the correct reasons for the large discrepancy in the numbers for the measured quantum requirement by these two pre-eminent

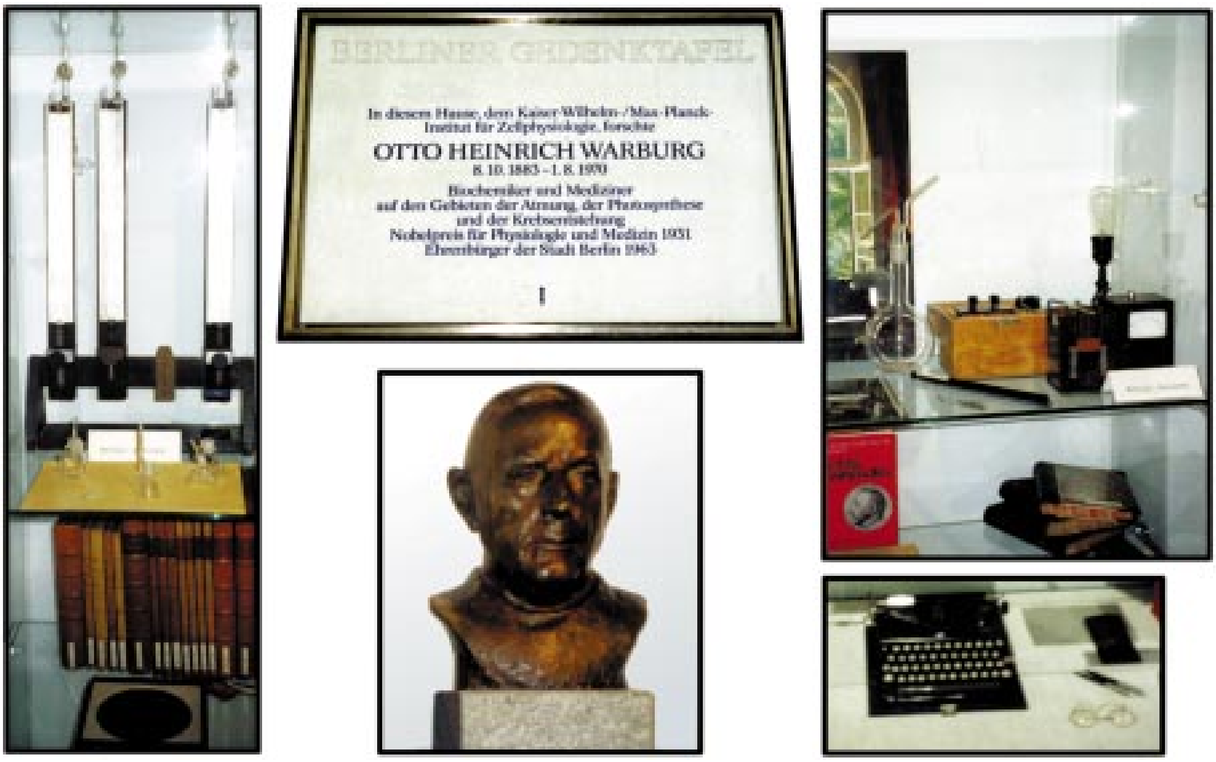

Figure 1.

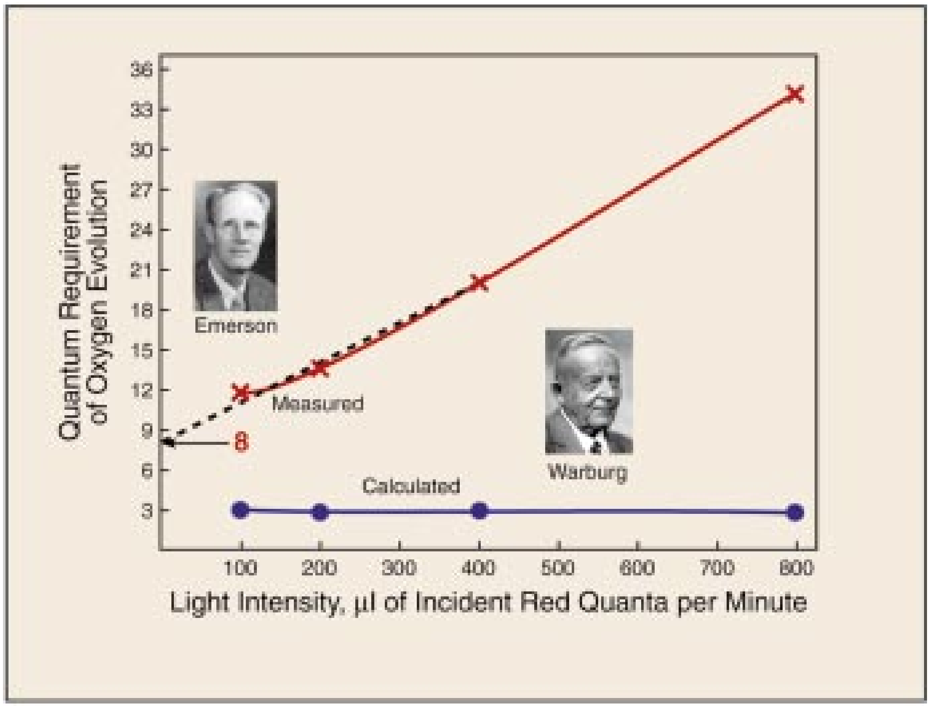

Figure 2.

authorities. It is worth noting that many experiments of Emerson were made with the same strain of the green alga *Chlorella* as used by Warburg, and many experiments were made under conditions identical to those used by Warburg. Further, both the investigators knew and used low intensities of light where the curve for the rate of photosynthesis versus light intensity is linear, and the quantum yield is maximal. In a large number of experiments by both the research groups, Warburg's carbonate-bicarbonate buffers were used to measure only oxygen, not carbon-dioxide (see a review by Emerson 1958). We emphasize that in 1969, Warburg et al. published, for the first time from their laboratory, a measured minimum quantum requirement of 12 per oxygen molecule released at the low light intensity. The importance of this paper is that it is the last and the final paper on this topic by Warburg. This measured value of 12 quanta per oxygen at low light intensities, if extrapolated to zero light intensity, could give a value of 8 quanta per oxygen evolution. The range of 8–12 is in full agreement with Emerson's values. However, Warburg did not believe that these high measured numbers are true numbers, and, presented calculated values of 3 to 4, using the *photolyte* concept (explained later in this letter). In our opinion, since 1969 there has been no difference in the reported experimental values of this number, and the controversy should have continued only on the basis of the correction factors that Warburg et al. (1969) applied to their results. This information does not appear to have been recognized by anyone and cannot be found in any research paper, any book or text book published to date. In view of the fact that both Warburg and Emerson were ideal experimentalists, this 'resolution' of the measured values brings relief to scientists at large. In this letter, a brief background of the controversy, and the reason why Warburg believed in 3 quanta per oxygen evolution is also presented. This should be of interest to all biologists as well as the text book authors in Biology, Biochemistry and Biophysics.

Warburg (1958) considered photosynthesis to be "like the world itself, nearly perfect." He wrote: "*It is a decision in favor of nature. The reaction by which nature transforms the energy of sunlight into chemical energy, and upon which the existence of the organic world is based, is not so imperfect that the greater part of the applied light energy is lost; on the contrary, the reaction is like the world itself, nearly perfect*." In other words, photosynthesis followed (Albert) Einstein's law of photochemical equivalency, i.e., one absorbed photon performed one photoact. This concept may have been reinforced in Otto Warburg by the work of his father Emil Warburg who confirmed this dictum in many photochemical reactions (Warburg 1918; Warburg and Rump 1928). It is important to mention here the *photolyte* hypothesis, that owed its origin partially in the mind of another Nobel-laureate of chemistry, Richard Wilstätter. To Warburg, it was a simple, ingenious and self-consistent scheme for photosynthesis: Warburg's experiments led him to believe that respiration is essential for photosynthesis. In his scheme, reoxidation of two-thirds of the carbohydrate produced was used to activate a chlorophyll-carbonic acid complex to give what was called a *photolyte* which could then be decomposed in a 'one quantum' process in conformity with Einstein's law, mentioned above. (Of course, the measured value could be lower than one, if the immediate product was partially degraded.) From this, and other details of their theory, then, Warburg surmised that the true overall minimum quantum requirement for one molecule of CO_2 fixed or O_2 released is 3! A simple calculation using a minimum need of 112 kilocalories when water and carbon-dioxide are converted to a moiety of carbohydrate and oxygen, and using red quanta (a mole

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Figure 1. Photographs of Warburg's manometers, vessels and record books (left); of the board in front of the building where Warburg worked in Berlin (middle, top); Warburg's bust in bronze (middle, bottom); of Warburg's bolometer (right, top); and of Warburg's typewriter, glasses and pens (right, bottom). Photographs by Govindjee.

Figure 2. Quantum requirement of the steady state oxygen evolution, as a function of light intensity, measured by Warburg et al. in 1969, in the green alga *Chlorella* (crosses): this measurement shows the number of quanta absorbed to release one oxygen molecule. The controversy between Otto Warburg and Robert Emerson had to do with the measured number at the low light intensity limit. Emerson (see photograph above the top curve) obtained a number of 8–12, and Warburg (photograph above the bottom curve) 2.8 to 4. It is obvious in the 1969 paper of Warburg et al. that their measured value was 12 at the lowest intensity they used, and the number could be 8, if extrapolated to zero light intensity (this author's dashed line and addition to the original figure). The circles, connected by the line, show quantum requirement of 3 calculated by Warburg and co-workers, using the amount of a hypothetical photolyte (carbonic acid bound to chlorophyll), which was also measured as oxygen release (for details, see Warburg et al. 1969). The abscissa simply is given in the unit of volume (μl) used by Warburg; in today's language, it should be converted into μmoles of photons.

of red photons, having about 40 kilocalories), would yield a minimum value of 2.8 quanta per oxygen molecule evolved. Photosynthesis would be then 100% efficient.

In 1923, Warburg and Negelein published their classic paper on the maximum quantum yield of oxygen evolution (its inverse being the minimum number of light quanta required to evolve one oxygen molecule). A minimum of ∼4 quanta per oxygen in the photosynthesis of the green alga *Chlorella pyrenoidosa* was measured by manometry that Warburg himself had perfected and which had become synonymous with his name (see Figure 1 for photographs of a bust of Warburg and equipment used in this work).

The first challenge

The first scientist to obtain a different result for the minimum quantum requirement was William Arnold (PhD thesis in 1935 at Harvard University, Cambridge, MA). Arnold later perfected his measurements during 1936–1937, at the University of California and the Hopkins Marine Station. He obtained a minimum value of 9–12 quanta per oxygen molecule, using the then novel technique of calorimetry. Arnold was a former undergraduate student of Robert Emerson, and co-discoverer of the concept of the *Photosynthetic unit* with Emerson (see Emerson and Arnold 1932a, b; Arnold 1991; Myers 1994). On the other hand, Emerson had done his PhD in the laboratory of Warburg in 1928. There was a reluctance by Arnold (perhaps, I might speculate, because Emerson could not believe at that time that his own Professor could be wrong, and, thus, did not think of encouraging Arnold) to publish this challenge to Warburg. It seems that in olden times, scientists had more reverence for their professors, and clearly Warburg was a major discoverer of biochemistry itself. Only at the insistence of Hans Gaffron was Arnold's work finally published in 1949 (see Arnold 1949, 1991 (p. 75, right column, lines 14–21); and Malkin and Fork 1996), but without any reference to the already raging controversy between Emerson and Warburg.

Controversy begins

The real challenge to Warburg's values came when Emerson and Lewis (1939, 1941, 1943a, b) finally attempted to check the 1938 observations of high quantum requirement (about 16 quanta per oxygen) by a highly prestigious group at Madison, Wisconsin that included a pioneer of photochemistry, Farrington Daniels (Manning et al. 1938). This was the beginning of the bitter controversy between Emerson and Warburg. Each time Emerson published a value close to 8–12, Warburg countered giving new conditions that Emerson must follow to obtain 2.8 to 4 quanta per oxygen molecule released (Emerson 1958; Walker 1993, pp. 135–141). This history will not be presented here. In an attempt to settle the controversy, Warburg was invited to the University of Illinois at Urbana, after World War II, to compare the experimental methods and materials. In spite of the fact that the laboratories in Urbana were maintained without heat during the winter months (on Warburg's order) and *Chlorella* cells were grown according to Warburg's recipes (except that there was no North window to grow the cells in Emerson's laboratory), each confirmed his own number. Neither party conceded, and the visit ended with bitterness on both sides instead of providing any solution to the problem (see Rabinowitch 1961).

After Emerson's death

Several years after Emerson's death on February 4, 1959, Warburg (1963) proposed 10% CO_2 must be provided to obtain the low quantum requirement. Warburg continued to deride Emerson's work; in a 1963 presentation at Gif-sur-Yvette, France, he told Eugene Rabinowitch (see p. 228 in Warburg 1963): "*Like many of Emerson's experiments, his discovery of the independence of the quantum yield from CO_2-pressure is wrong*." Warburg (1958) had also discussed that blue catalytic light is necessary for the low quantum requirement. Thus, in 1968, Rajni Govindjee, Eugene Rabinowitch and Govindjee repeated Warburg's experiments using young synchronous cultures of algae, 10% CO_2 and blue catalytic light. R. Govindjee et al. (1968) confirmed Emerson's, not Warburg's values.

The 1969 paper of Warburg

It has been generally believed that Warburg had always obtained a minimum value of 2.8 to 4 quanta per oxygen and many have sought to understand this discrepancy – with as many ideas as the number of investigators. We note here that Warburg et al. (1969) themselves measured a value of 12 quanta per oxygen at the lowest light intensity they used (see Figure 2). (If one extrapolates to zero intensity, a value of 8 quanta per oxygen could be calculated; this, however, is of no particular importance to the conclusion of this letter.) Thus, there has not been an experimental discrepancy since 1969 between Warburg's measured value of 12 and those of any other investigator including Emerson (8–12). However, this 1969 paper of Warburg and coworkers seems to have escaped detection or, at least, critical reading by most investigators and book writers in photosynthesis.

After reading the 1969 paper of Warburg et al., it appears that Warburg's major concern was that the values of 8–12, obtained by Emerson and others, somehow contradicted Einstein's law of photochemical equivalence (one photon leading to one photochemical event). If Warburg had accepted that in photosynthesis, two light reactions drive the transfer of four electrons from water to carbon dioxide in two steps (8 primary events) (Hill and Bendall 1960; Duysens 1989 for history), he would have had no difficulty in accepting the measured minimum quantum requirement of 8. Instead, Warburg et al. (1969) argued that their measured value of 12 is not a true value of the quantum requirement. They obtained values of 3–4 using a circular reasoning: they calculated the fraction of the light absorbed from oxygen measurements from a totally hypothetical and unsubstantiated photolyte (chlorophyll bound to carbonic acid). This was then used to conclude "the quantum requirement of the splitting of the photolyte is always 1" which Warburg believed was "as required by Einstein's law". Warburg et al. (1969) further stated "*We do not hesitate to express here our satisfaction that after the short time of 46 years (Warburg and Negelein 1923) truth has now won its war also in the main reaction of bioenergetics*". It seems that there was no real War after the 1969 paper. The authors failed to emphasize that their new measurements were in agreement with the measurements of Emerson. No one had doubted the validity of Einstein's law: the two light reaction scheme is in agreement with the measured quantum requirement values of 8 at low light intensities not only of Emerson and coworkers (including Arnold 1949; and R. Govindjee et al. 1968), but, we emphasize, *Warburg himself*.

Thus, no controversy need be considered to exist between Warburg's measured values and those of the others. The photolyte has not been confirmed or supported by any investigator; also, the quantum requirement of 3–4 is not consistent with the current two-light reaction, two -photosystem scheme of photosynthesis (Hill and Bendall 1960; Duysens 1989). The current scheme of photosynthesis could record a minimum quantum requirement of 4 *only* under the following conditions. (1) Transient oxygen evolution involving only Photosystem II were to be measured, and (2) Quanta absorbed only in Photosystem II were counted. This is easy to understand since Joliot et al. (1969) had demonstrated the period 4 oscillation in a plot of oxygen released per flash of light as a function of flash number. The existence of a plastoquinone pool between Photosystems II and I in dark-adapted *intact* photosynthesizing cells would allow this oxygen yield to be measured for sometime. But, then, corrections would have to be made for quanta absorbed in Photosystem I to get a quantum requirement of 4. Warburg and Negelein's 1923 results, and Warburg et al.'s 1969 calculations of 3–4 quanta per oxygen could have become understandable only if they could be related to Photosystem II alone. But, this is *not* what Warburg and Emerson had measured. They measured, instead, quantum requirement of steady state oxygen evolution with both photosystems present, and without any corrections for quanta absorbed by Photosystem I. In conclusion, then, we should remember that Warburg himself, in his last paper in 1969, just before his death in 1970, confirmed Emerson's measured value of minimum quantum requirement for oxygen evolution.

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