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Review Article

THE GREATEST SECRET OF PHOTOSYNTHESIS

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Summary: The investigations of the photosynthetic oxygen production reaction induced by separated groups of 4 flashes have shown that during the induction time of photosynthesis (i.e. during the dark to light transition time) an extremely fast and intensive process of blocking of the oxygen evolving centres occurs. Contrary to the generally accepted concepts, the estimations lead to the conclusion that the number of the blocked centres under saturating irradiance conditions is of the order of the number of chlorophyll molecules in the investigated samples, and from 600 oxygen evolving centres approximately only 1 remains in an effective state. These findings explain the results obtained from the classic experiments of Emerson and Arnold without using the notion about the photosynthetic units and finally lead to the conclusion that the contemporarily accepted two fundamental concepts, the concept of photosynthetic units and the concept of the two photosystems or Z-scheme, have been postulated neglecting the principle of sufficient argumentation.

Keywords: Photosynthesis; Fundamental concepts; Photosynthetic units; Z-scheme.

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INTRODUCTION

As a PhD student at the Moscow State University in 1967, I was extremely impressed after getting familiar with the existence of structures like the photosynthetic units in the photosynthesizing systems (Emerson and Arnold 1932a, b; Gaffron and Wohl, 1936). I started considering several possible hypotheses, from a physical point of view, for such a directed energy transfer from hundreds of similar molecules to 1 "specialized", called "reaction centre", a molecule of the same type, which is able to perform the photochemical charge separation reaction. Finally, I came to the conclusion that the only possible mechanism for such kind of events could be the existence of a demon analogous to Maxwell's Demon in Thermodynamics. So, I began investigating the experimental basis of this extremely interesting and widely accepted concept.

In early 1932, Emerson and Arnold applied flash-induced experiments in photosynthesis for the first time, using manometric equipment, introduced by

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Warburg, and saturating flashing light, obtained by the discharge of a 1 or 0.5 μF condenser charged to about 3000 Volts, through a neon tube[1]. The main important observations from these investigations are well known and could be shortly described as follows:

1. The average flash yields (CO_2 reduction or O_2 evolution) were maximal when the spacing (dark period) between the flashes was about 0.02 s (20 ms). This means that the time required for one unit in the photosynthetic machinery to complete the cycle of photochemical and Blackman (dark) reaction was around 20 ms at 25°C.

2. The dependence of the quantity of the oxygen molecules in moles, evolved after one saturated flash on the number of the chlorophyll molecules in *Chlorella* cell suspensions was linear (Fig. 1) with a slope of about 1 O_2/2480 chlorophylls, thus suggesting that after every flash 1 oxygen molecule was produced by 2480 chlorophyll molecules. According to Emerson and Arnold (1932a, b) this result was considered as the crucial experimental fact supporting the idea of the existence of the structures like the photosynthetic unit.

3. A good coincidence was observed between the experimentally obtained maximal rate of oxygen evolution, P_{max}, under saturated continuous irradiation and the theoretically calculated maximal rate, $P = N/\tau$, where N is the number of the oxygen producing units (reaction centres) and τ is the turnover time estimated from flash light experiments. This correlation showed that under continuous saturating irradiation the number of the effectively operating oxygen evolving centres was approximately 1/600 of the number of chlorophyll molecules in the investigated suspension, as estimated in the flash experiments.

In 1932, besides these important observations obtained by Emerson and Arnold, some additional facts were also recognised:

4. The very high (maximum) quantum efficiency of photosynthesis under limited light intensity conditions, i.e. when the probability for light quanta absorption of a chlorophyll molecule was about 1 quantum per hour.

This statement is a direct consequence of the investigations of the light (irradiance) dependence of photosynthesis. It has been shown in many experiments that the photosynthetic response to very low light intensities is linear (see Part I, Fig. 1.1, curve “A” and the relevant text in Zeinalov, 2010). This type of light curves was accepted as a general rule.

5. The absence of a noticeable induction time in the process of oxygen evolution or CO_2 reduction under very low light intensity conditions - this means that at low irradiances photosynthesis starts before the absorption of 4 quanta, needed for the evolution of one O_2 molecule, by separated chlorophyll molecules.

A detailed analysis of these extremely important observations and experimental results has been presented in my works (Zeinalov 2009b; 2010). In the present paper, we have focused on the main unproven assumption made by Emerson and Arnold (1932a, b). In their experimental setup (the manometric equipment introduced by Warburg) they had no possibility to track the O_2 yields per individual flashes. They had to illuminate a suspension of unicellular algae with

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[1] It is clear, that at that time the oxygen rate electrode and the photographic flash tubes were not available.

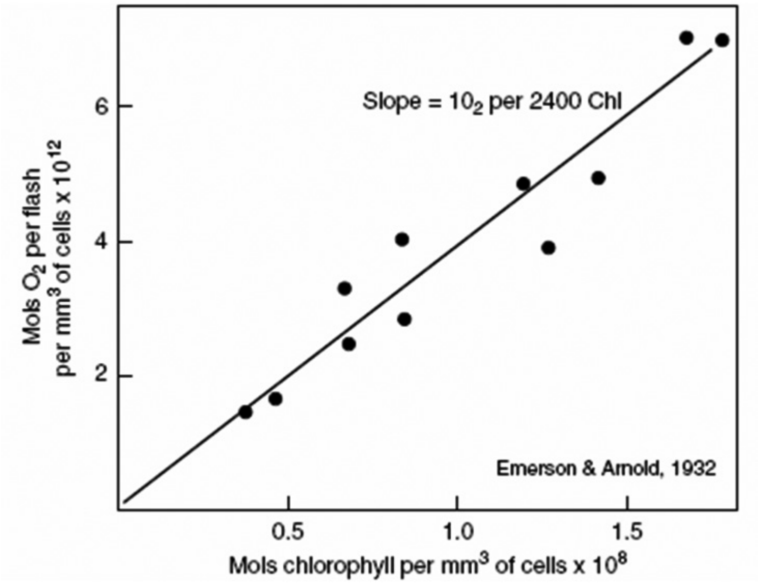

Figure 1. Dependence of the amount of oxygen molecules evolved per saturated short flash on the number of chlorophyll molecules in a suspension of *Chlorella*. Data by Emerson and Arnold (1932a, b).

thousands of flashes and after estimating the total number of evolved oxygen molecules (using the increase of the pressure in the manometric vessels) they could calculate the average oxygen flash yield. Let us point out that since then (1932) up to the present (2016) there has been no clear understanding of the processes occurring after switching the light on, i.e. during the induction time of photosynthesis. Emerson and Arnold (1932a; b) simply assumed that the number of effectively working oxygen evolving centers during the dark to light transient time remained unchanged. The results presented in this work as well in our preceding publications (Zeinalov 2009a, b and c) demonstrate and prove that this assumption is wrong.

MATERIALS AND METHODS

The experiments were performed with *Chlorella* or *Scenedesmus* cell suspensions using the equipment described in a previous publication (Zeinalov, 2002). The equipment consisted of a polarographic oxygen rate electrode and a system for producing 4 saturating flashes which could be ignited in a group with different timing between each one (between the flashes) and with variable dark time intervals between the groups. This equipment was specially designed for estimation of the number of open (functionally active) reaction centres under different conditions.

RESULTS

The concept of the photosynthetic units – a wrongly introduced postulate

Considering the integral equation of photosynthesis:

$$CO_2 + H_2O + h\nu \rightarrow (CH_2O) + O_2$$

it could be seen that for the reduction of one CO_2 molecule to the level of carbohydrate, 4 electrons should be transferred on account of the absorbed light quanta energy. If we consider the

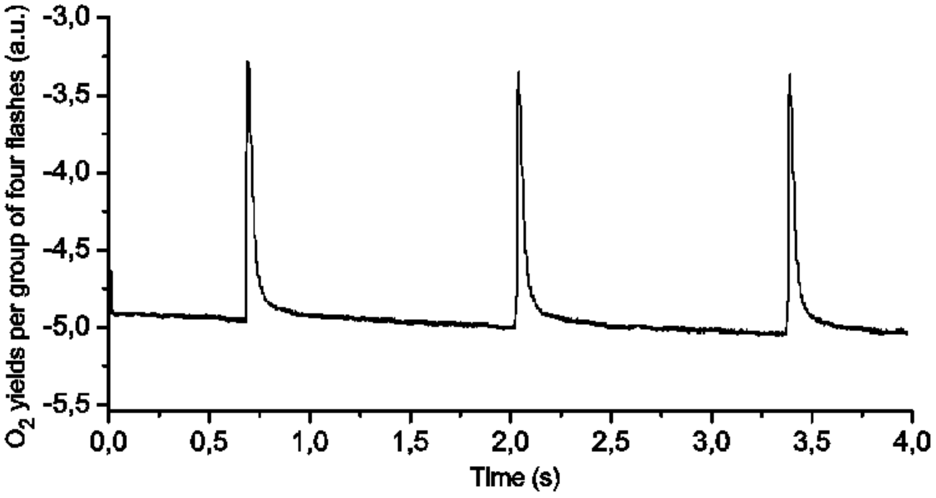

Figure 2. Oxygen yields produced by groups of 4 and short (10 µs) saturating flashes, spaced with 20 ms between each other and 1.3 s between the groups. The three flash groups were applied after 5-min dark incubation of a suspension of *Chlorella pyrenoidosa.*

generally accepted Kok's scheme (Kok et al., 1970) we will see that independently from the starting state (S_i – state) any oxygen evolving center after accepting 4 light quanta, spaced at least 20 ms apart, will evolve one O_2 molecule and will return to the initial starting S_i state. This means that if the 4 saturating and short (several µs) flashes are applied in darkness (all centres are in an open state), the amplitudes of the O_2 bursts registered by the oxygen rate electrode will be proportional to the number of the oxygen evolving centers in the investigated algae or chloroplast suspension. A triad of O_2 yields produced by three groups of 4 flashes, individual flashes spaced 20 ms apart, with 1.3 s between the groups, from a suspension of *Chlorella pyrenoidosa,* is shown in Fig. 2. As was mentioned above, the amplitudes of the bursts should be taken as proportional to the number of oxygen evolving centers in relative units[2].

The oxygen yields produced by separated flash groups depending on the spacing between flashes are presented in Fig. 3. Our results showed that the yields increased with increasing the spacing between the flashes in the groups within the interval 0.1 ms (100 µs) - 10 ms and after that (up to 1 s) the yields decreased. Thus, the maximum yields should be registered between 10 ms and 20 ms. The presented data confirm the finding of Emerson and Arnold (1932a; b) that the turnover time of the oxygen evolving centers is around 20 ms at 25^0 C.

The secret of the induction phenomena

Let us consider the so-called induction phenomena, i.e. the changes in

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[2] As I have shown (Zeinalov 2010) this statement is not entirely correct. In darkness part of the produced O_2 by the flash groups is used for dark respiration, so the amplitudes of the flash group yields are partly reduced. Nevertheless, the amplitudes of the flash groups could be approximately used for estimation of the total number (in relative units) of oxygen evolving centres.

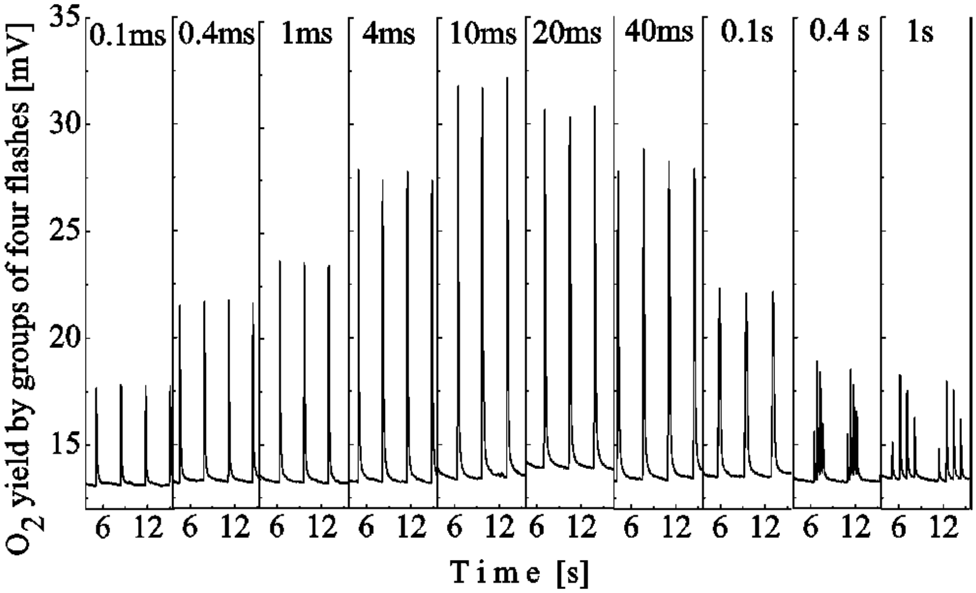


Figure 3. Steady state oxygen yields of groups of 4 saturating flashes depending on the spacing (from 0.1 ms up to 1 s indicated on the graphs) between the flashes in the groups in a *Scenedesmus obliquus* suspension with absorbance 0.05 (0.1 ml sample volume). The groups of 4 saturating flashes (4J, $t_{1/2}$ = 10 μs) are spaced at 3 s.

the rate of photosynthesis after irradiation of a dark adapted photosynthesizing system with continuous light. In 1939 Emerson and Lewis observed changes in the photosynthetic O_2 and CO_2 exchange after the dark-to-light and light-to-dark transients (Emerson and Lewis, 1939). The observed CO_2 burst after switching on the exciting light (during the induction time of photosynthesis) is known as Emerson's first effect.

In Fig. 4 a typical oxygen induction curve is presented using a suspension of *Chlorella pyrenoidosa* and an oxygen rate electrode as described by Zeinalov (2002). It is obvious that after switching on the light one very sharp oxygen burst (a) was observed followed by some oscillations (2 minimums – b and d, and 1 maximum c) before reaching a steady state rate. On the other hand, after switching off the light, i.e. in darkness, the oxygen evolution rate sharply decreased (the operation of Kok's model mechanism is switched off (stopped)) without reaching the zero level, followed by a second slow component with a low rate constant.

Now the essential question arises: what happens during the induction time, so that the oxygen induction curves have such a complex character? There are two well known phenomena (effects): the first effect of Emerson, i.e. the CO_2 burst after switching on the exciting light and Warburg's oxygen effect, i.e. the inhibitory action of O_2 on photosynthesis.

The following two statements should be accepted without any objection: i) if the flash groups of 4 flashes spaced with 20 ms are given on the background of saturating continuous irradiation, the amplitude of the oxygen bursts produced by the groups will be zero, since all oxygen evolving centres are in a closed (working) state; ii) since

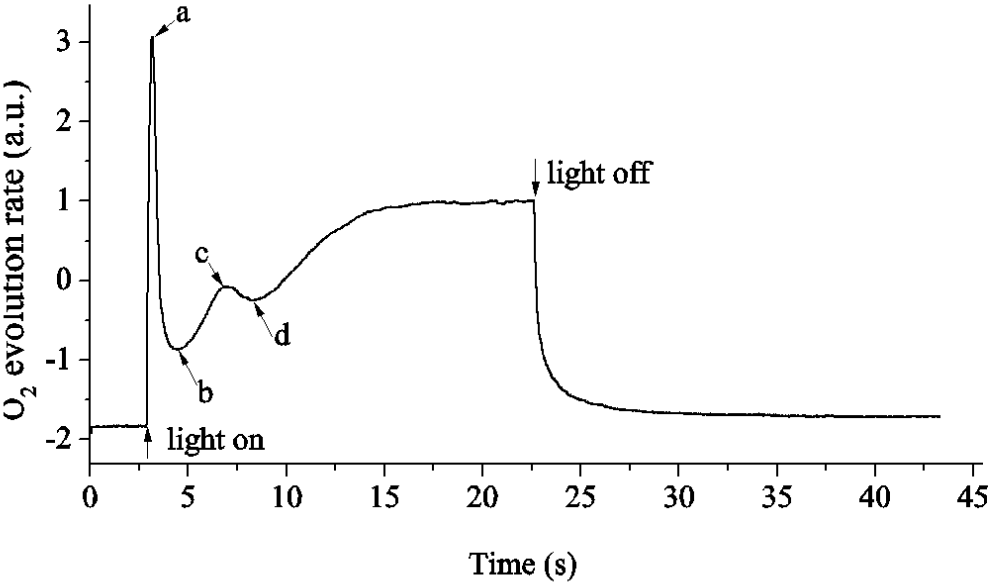


Figure 4. A typical induction curve registered with an oxygen rate electrode in a *Chlorella pyrenoidosa* suspension (0.1 ml sample volume, 15 µg Chl) after 5 min adaptation in darkness. A saturated (400 µmole photons.m^{-2}.s^{-1}) monochromatic (650 nm) light beam was applied.

the turnover time of the reaction centers is 20 ms, the amplitudes of the oxygen yield bursts produced by the groups of flashes 20 ms after switching off the continuous saturating irradiation should reflect the number of oxygen evolving centres which are operative (functionally effective) under irradiation with continuous light. According to the founders of the concept of photosynthetic units, the last number should be equal to the number obtained in darkness, i.e. before irradiation with continuous light. This statement is obviously wrong (compare the amplitudes of the 5 flash group yields in darkness before switching on the continuous irradiation (Fig. 5) with the amplitudes of the first flash group, which is produced 12.5 s after switching off the continuous light)! Contrary to our expectations the amplitudes of the yields are invisible; and only after 30 s amplitudes could be observed, slowly increasing after a longer period in darkness. However, even after 10 min these amplitudes did not reach the initial starting amplitudes (For more details, see Zeinalov, 1982 and 2009a).

As was mentioned above, the amplitudes of the oxygen bursts should be accepted as reflecting the number of open reaction centres, i.e. if the groups of 4 flashes are given after 20 – 30 min adaptation in darkness (pay attention to the first 5 bursts as shown in Fig. 5) the amplitude should reflect the total number of reaction centers (RCs). According to the concept of photosynthetic units and my recent experimental data the closed (working) state time of the RCs is in the order of 10 – 20 ms (Fig. 3). This means that after switching off the continuous light the amplitude of the flash group yields, even only after 20 ms should be equal to the initial amplitudes before switching on

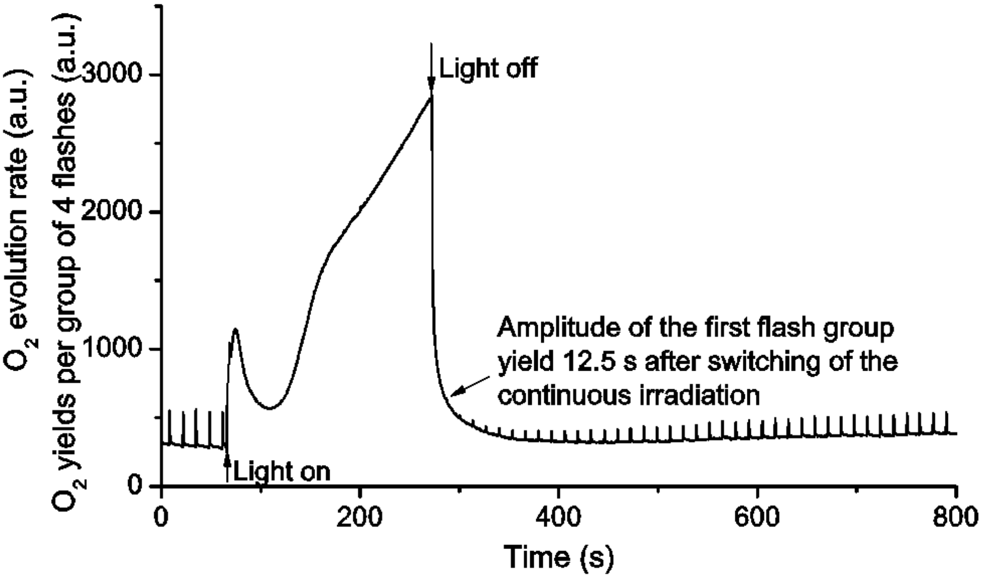


Figure 5. Variations in the amplitudes of oxygen bursts produced by groups of 4 saturating (4 J, $t_{1/2}$ = 10 ms) flashes before, during and after the induction time of photosynthesis in *Chlorella pyrenoidosa,* (0.1 cm^3 sample volume, 15 µg Chl) registered with an oxygen rate electrode, as described by Zeinalov (2002). The suspension of cells was kept in darkness for 30 min and the groups of 4 saturating flashes (20 ms spacing between the flashes and 15 s between the groups) were switched on at "0" time point. The saturating (650 nm monochromatic light beam with 400 µmole photons.m^{-2}.s^{-1}) was switched on at the time indicated by „↑" and switched off at the time indicated by "↓ ".

the continuous irradiation. This statement is obviously wrong (see the amplitude of the first flash group in Fig. 5, which was produced 12.5 s after switching off the continuous irradiation)!

The analysis of the obtained results leads to the conclusion that during the induction time of photosynthesis, i.e. during the dark-to-light transient time of a given photosynthesising system (e.g. *Chlorella* cells suspension), an extremely fast process of inactivation (blocking) of the essential part of the oxygen evolving centres occurs.

Obviously, even after such a short time (110 ms) of irradiation with continuous light, the centres were partially blocked (compare the amplitude of the first oxygen burst produced by a group of 4 flashes after switching off the continuous irradiation with the amplitudes before switching on the continuous irradiation) (Fig. 6). However, if the irradiation time with continuous light was decreased below 40 ms (Fig. 7) the amplitudes of the yields produced from flash groups remained unchanged. The estimations (Zeinalov 2009b) led to the conclusion that only 1 out of 600 reaction centres remained approximately active under saturating continuous irradiation. In reality, using the manometric equipment of Warburg, Emerson and Arnold (1932a, b) have determined only the number of unblocked, functionally active reaction

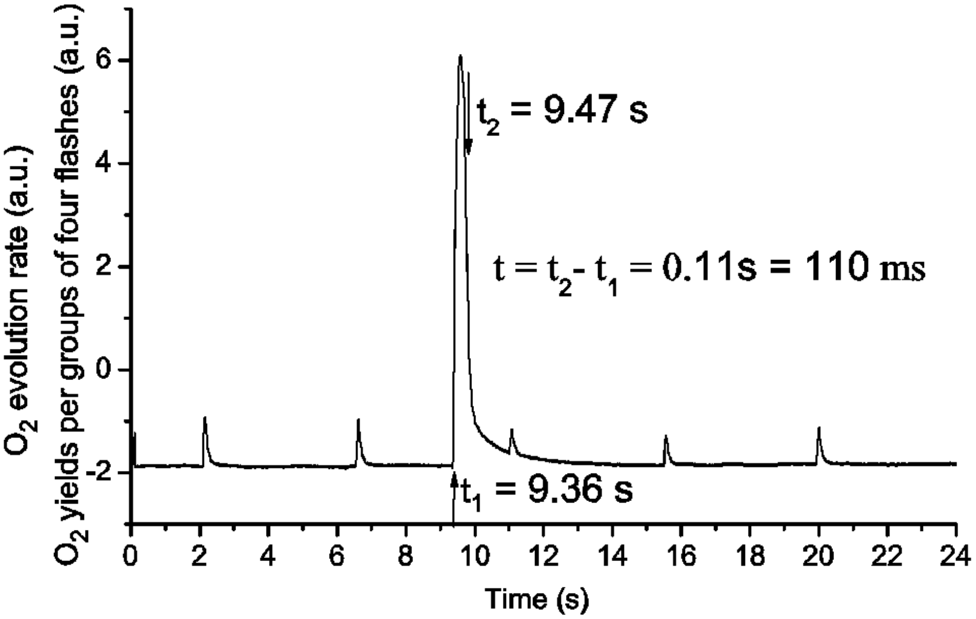


Figure 6. The same experiment as shown in Fig. 5 except for the duration of the induction time (irradiation with continuous saturating light was decreased to 110 ms).

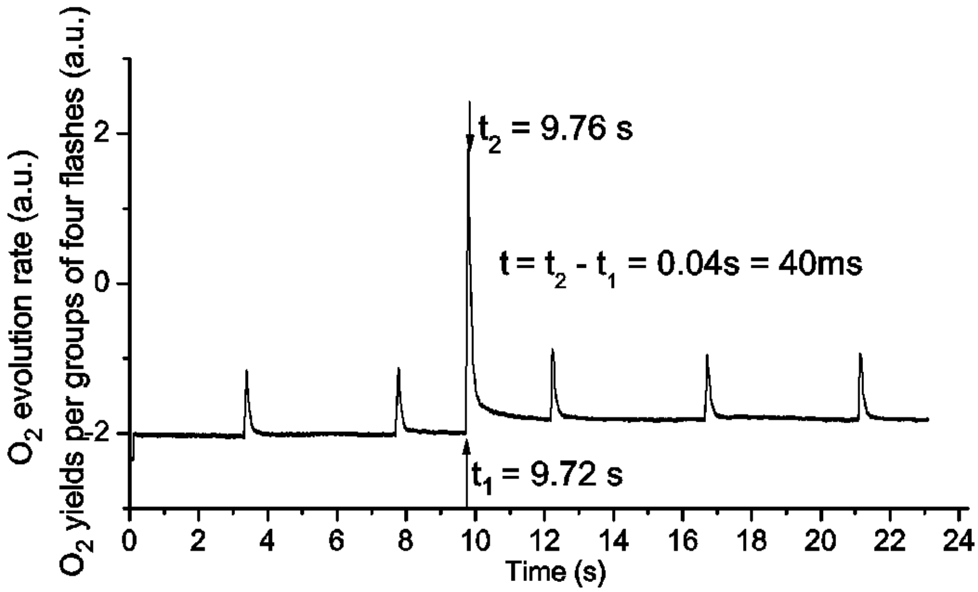


Figure 7. The absence of inactivation of the oxygen evolving centers under continuous irradiation with saturating light after decreasing the irradiation time below 40 ms (for details see the text).

centres under saturating irradiance condition (the irradiation with saturating light flashes, spaced at 20 ms as used in the Emerson and Arnold's experiments is approximately identical to the effect of saturating continuous irradiation!).

Taking into account different phenomena during the induction time of photosynthesis, (Zeinalov 2009a), it could be estimated that the number of blocked reaction centres under saturating irradiance conditions is in the order of the number of chlorophyll molecules in the investigated suspensions.Thus, a conclusion can be made that the total number of reaction centres (in darkness) is equal to the number of chlorophyll molecules. This means that under very low irradiances every chlorophyll molecule is in a state to perform the charge separation reaction and consequently there is no discrimination between these molecules. In other words, we should reject the widely accepted statement that some of the molecules are playing light harvesting (antenna) role and only a limited number of them are in a state to perform photochemical reactions (Emerson and Arnold, 1932a, b; Gaffron and Wohl, 1936).

The changes in the amplitudes of oxygen bursts under irradiation with non-saturating continuous light (100 µmole photons.m^{-2}.s^{-1}) are presented in Fig. 8. The data showed that even under significantly low light intensity (compare the amplitudes of the bursts on the induction curves with the amplitudes of the initial bursts, i.e. in darkness) the reduction of the burst amplitudes after switching off the light (in darkness) was apparent. Another interesting phenomenon in this case was

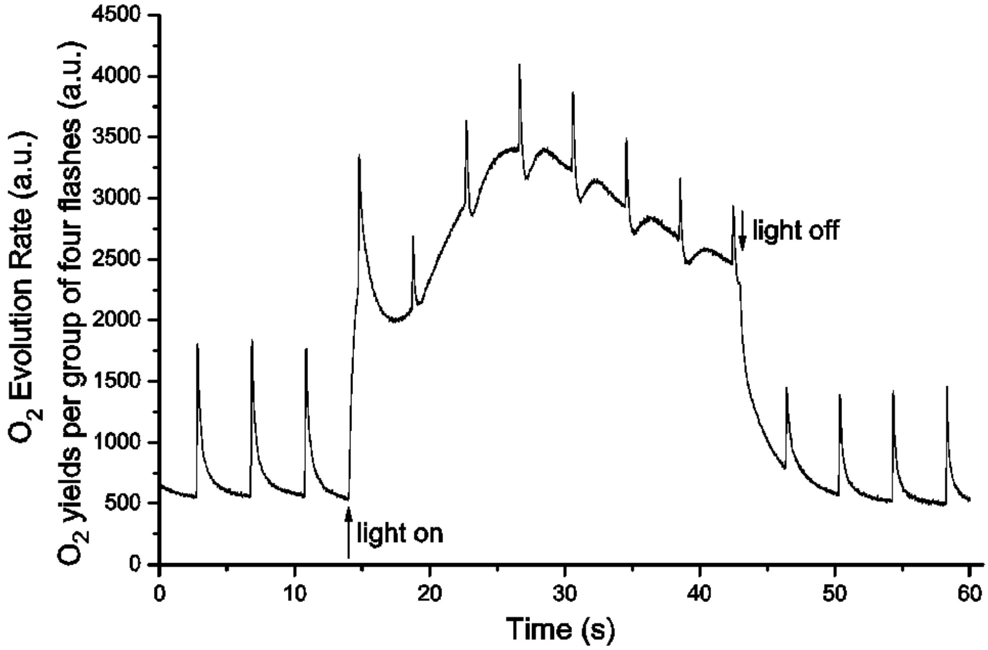


Figure 8. Changes in the amplitudes of the oxygen bursts produced from groups of 4 flashes under continuous irradiation with non-saturating light (100 µmole photons.m^{-2}.s^{-1}) (for details see the text).

the observed decrease in the oxygen evolution rate during the induction time after every flash group. Obviously these decreases are related to the oxygen absorption reaction leading to the auto-regulation of the number of effectively working oxygen evolving molecules, and in this way to the regulation of the photosynthetic process itself, thereby retaining an optimal O_2 concentration in the living cell and chloroplast volumes.

This is the only way to promptly regulate the photosynthetic intensity and O_2 concentration in the living cells or chloroplasts after sudden changes in the light intensity during the day – e.g., a transition between cloudy and sunny weather within in a few seconds. In this respect, it should be noted that Mother Nature has taken all precautions in advance. The assumption that the intensity of photosynthesis is regulated by the rate of the dark reactions (Calvin-Benson cycle), taking part after the light-induced reactions, i.e. with the participation of $NDPH_2$ and ATP, could refer only to the slow changes observed in photosynthesis during the day. This means that the fast changes in the intensity of photosynthesis (e.g. during the induction time) are regulated by O_2 concentration levels in the volumes of the thylakoid and chloroplasts.

The simple logic leads us to the following statement: If CO_2 is evolved after switching on the light, which is shown by Emerson's first effect, then CO_2 should be absorbed during the dark incubation time! On the other hand, it is seen in the presented graphs, that the oxygen evolution rate does not decrease to the dark level immediately after switching off the light and the oxygen evolution continues for prolonged time in darkness. The only logical assumption is that during the induction time, i.e. after switching on the light, a significant amount of oxygen is absorbed (accumulated) somewhere! As it is known from the data reported by Emerson and Lewis (1941 a; b) as well as McAlister (1939), the amount of CO_2 burst during the induction time is in the order of chlorophyll molecules in the investigated algae suspensions. Therefore, in darkness every chlorophyll molecule, most probably in a complex with glutamate, catches a CO_2 molecule (or HCO_3^-).

The concept of two photosystems – the second mistake of Emerson

If the concept of photosynthetic units is wrongly postulated, this leads to the logical conclusion that the concept of two photosystems or the widely accepted Z-scheme is also a mistake. No one could imagine that every two chlorophyll molecules are equipped with a complex electron transfer chain (Z-scheme). A careful analysis of the fundamental observations included in the formulation of this concept shows that the only observation to be explained based on the postulation of the two different photochemical systems is the "Enhancement" phenomenon, or "Emerson's second effect". All other experimental results, explained during the years using the Z-scheme, could be explained without this concept. However, our investigations (Zeinalov 2009b, c) showed that the enhancement effect was a simple consequence of the non-linearity of the photosynthetic light curves under low irradiance conditions. The non-linearity is produced by the operation of the non-cooperative (Kok's) mechanism, as well as the dark respiration, and the process of blocking the oxygen evolving centres. In reality, the analysis of the data presented in Fig. 9A clearly shows that the observed decrease of the quantum efficiency in the far red region (after the absorbance maximum at 680 nm) i.e. the "Red drop effect" and the following increase (after applying the additional irradiation - "Enhancement effect") are simply a consequence of the non-linearity of the light curves under low irradiances (compare with Fig. 9B). Moreover, the significant increase in the value of quantum efficiency between 660 nm and 680 nm under irradiation with two light beams with equal wavelengths (Fig. 9A), which cannot be explained with the Z-scheme, clearly proves the existence of non-linearity of the light curves in the experiments conducted by Emerson et al. (1957) and Govindjee (1963; 2000).

Mitohondrial and thylakoid membranes – an analogy

While water is still thought of as an immediate electron donor to the reaction centres of photosynthesis (with 2 molecules of water simultaneously submitted to a complex cleavage reaction), it seems that CO_2 or more likely the bicarbonate ion (HCO_3^-) is more suitable to play this role. If this is the case, then the answer to the question "What is the nature of the oxygen evolving reaction – electrolysis, photolysis or radiolysis" should be **electrolysis**. Consequently, the problem of the special structure of the reaction centres (see Renger 1997; Hoganson and Babcock

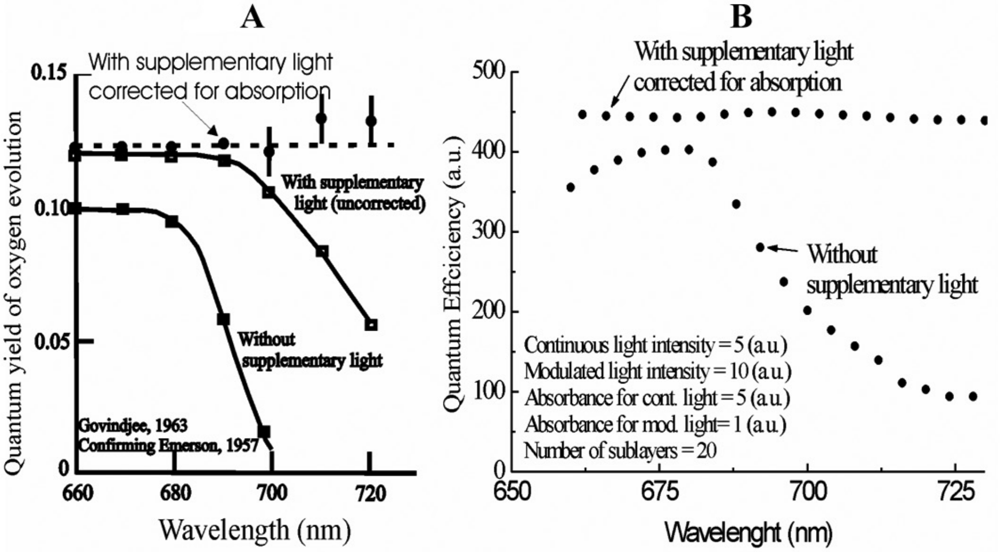


Figure 9. Experimentally obtained results by Govindjee (1963; 2000) for the "Red drop" and the "Enhancement" effecs of Emerson (1957) on the quantum efficiency of photosynthesis (A) and theoretically calculated results using the non-linearity of the photosynthetic light curves (B). As it could be seen the "Enhancement effect" is explained entirely with the non-linearity of the light curves and suspension absorbance properties (for details see Zeinalov, 2009c; 2010).

1997; Tommos and Babcock 1998; Raval et al., 2005) for the realization of water photolysis should be dismissed. It could be speculated that the absorbed light quanta energy by chlorophyll molecules, transferred into the energy of electric field (as a potential difference (around 1.2V!) between the inside and outside thylakoid membrane) is used for HCO_3^- and H^+ electrolysis. In this aspect, the analogy between the functions of mitochondrial membranes and thylakoid membranes seems more complete. The main difference is in the mechanisms of membrane electrifying (Fig. 10). According to the Mitchell's hypothesis, it is clear that the phosphorylation needs: i. Energy of electric fields; ii. pH gradient; iii. Structure, like the membrane, which will support the electric field and the pH gradient; iv. Mechanisms and energy sources (organic fuels) for creating the electric field and pH gradient; v. Special apparatus for synthesis of ATP - ATP-synthase.

A comparison of the two matchless membranes, mitochondrial and thylakoid, yields the following speculation. All parts of the mitochondrial membrane with ATP-synthase, participating in ATP synthesis, are situated in the thylakoid membrane. The only principal difference is the absence of organic fuel for creating the electric field and pH gradient. Instead, the light or the energy of the excited chlorophyll molecule is transformed into an electric field. The protons inside the thylakoid are generated through decomposition of H_2CO_3 either with the aid of carboanhydrase or by a special mechanism involving the participation of plastoquinone.

The negatively charged outside the membrane surface should have sufficiently high possibility to donate electrons to different compounds present in the reducing side of the so-called "Z scheme". This may explain why some of the biochemical investigations have shown that $NADP^+$ is reduced by the so-called PSI, while others (Arnon et al., 1980) have pointed out that the same reaction is performed by PSII, and this is the reason for the existence of several contradictory hypotheses about the sequence and functioning of the two light reactions of photosynthesis (Hill and Bendall, 1960; Knaff and Arnon, 1969; Park and Sane, 1971; Huzisige and Takimoto, 1974; Arnon et al., 1980). In principle, the effects of both "photosystems" from a physical point of view are identical - electron transfer from the lumen side of the thylakoid membrane to the stroma side, i.e. membrane electrification. In this sense the term "photosystem" should be used for the entire thylakoid membrane operating as a complete photovoltaic system.

Mechanisms of the photosynthetic oxygen producing reactions

Having in mind the statement of Vennesland (1966), that the molar equivalent relation of the aerobically bound CO_2:glutamate:chlorophyll is 1:1:1 showing that all these components contribute to the activated complex which functions as an O_2 precursor when it absorbs light, we should accept that the dark state of photosynthetic oxygen evolving centres should be presented by the expression $Chl.GM.HCO_3^-$. This state is presented by the S_0 state in the original Kok's scheme, where Chl is chlorophyll,

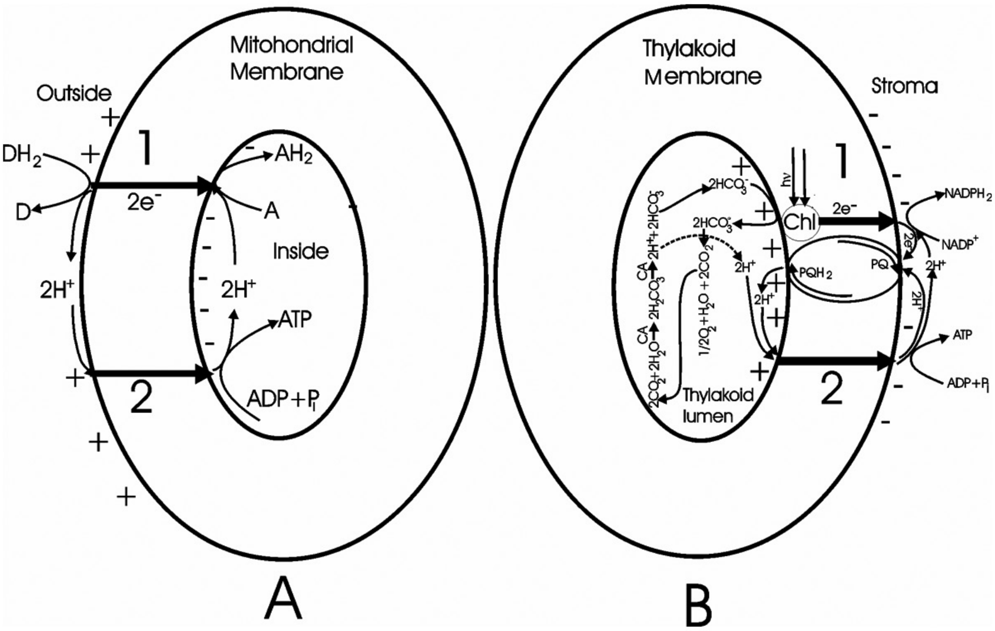
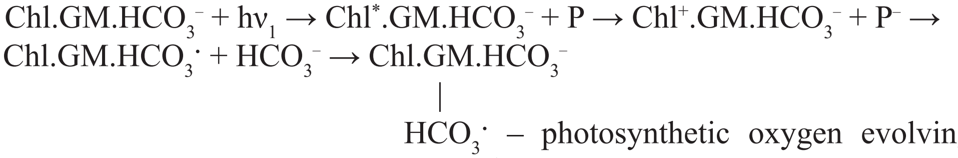


Figure 10. A – Chemiosmotic hypothesis of Mitchell (1961) for mitochondrial membrane. The donor of hydrogen DH_2 is oxidized by electron carriers outside of the membrane. As a result, $2H^+$ remain outside of the membrane and $2e^-$ are transported to the other side. The electron carriers localized at that place deliver the electrons to an electron acceptor A, which incorporates $2H^+$ from the internal aquatic phase. So, the oxidation of one molecule DH_2 by A leads to the evolution of $2H^+$ outside and the absorption of $2H^+$ in the internal space (section 1). In this way, an electric field arises between the membrane sides. At the mitochondrial membrane, the donor of H^+ is ascorbat (DH_2). The electrons are transferred from the outside to the inside of the membrane through the respiratory chain, oriented across the membrane on cytochromoxidase and the electrons are transferred to the acceptor A, in this case O_2. The process of phosphorylation (section 2) leads to the movement of H^+ in a direction opposite to that of respiration. The synthesis of 1 ATP from ADP and P_i is connected with the evolution of $2H^+$ in the matrix and absorption of $2H^+$ outside. In this way, the mechanism of phosphorylation uses the electrochemical H^+ gradient created by the functioning of the respiratory chain.
B – Processes carried out in the thylakoid membrane according to the concepts proposed by Zeinalov (2010). In contrast to mitochondria, electric field induction is a process created by excitation of the chlorophyll molecule by the light (section 1). The acceptor of electrons is $NADP^+$ (instead of O_2 during mitochondrial respiration) and PQ pool, included in the cyclic electron transport chain (see also Fig. 5.3 in Zeinalov, 2010). The donor of electrons is HCO^-_3 (bicarbonate ions, instead of ascorbat). The remaining parts of the figure could be compared with the sequence of reactions (4.8) – (4.14) presented in Part IV of my book (2010).

GM is glutamate and HCO_3^- is bicarbonate ion. This state after receiving 1 light quantum (hν) should be described by the following expression:

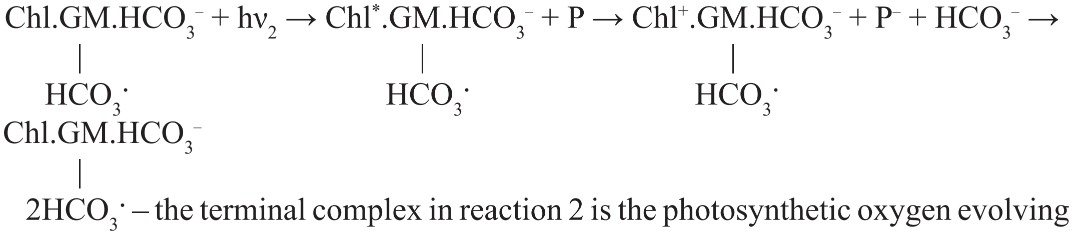
1.



$$\mathrm{Chl.GM.HCO_3^- + h\nu_1 \rightarrow Chl^*.GM.HCO_3^- + P \rightarrow Chl^+.GM.HCO_3^- + P^- \rightarrow Chl.GM.HCO_3^{\cdot} + HCO_3^- \rightarrow Chl.GM.\underset{\overset{|}{HCO_3^{\cdot}}}{HCO_3^-}}$$

– photosynthetic oxygen evolving centre after receiving the first photon; Chl* – excited state and Chl^+ – oxidized state of Chl molecule; P – the electron acceptor on the outside surface of the thylakoid membrane. This state reflects the S_1 state in the Kok's model. HCO_3^- is the bicarbonate ion and $HCO_3^{\cdot}$ is its radical shape. This state after receiving the second light quantum will proceed as follows:

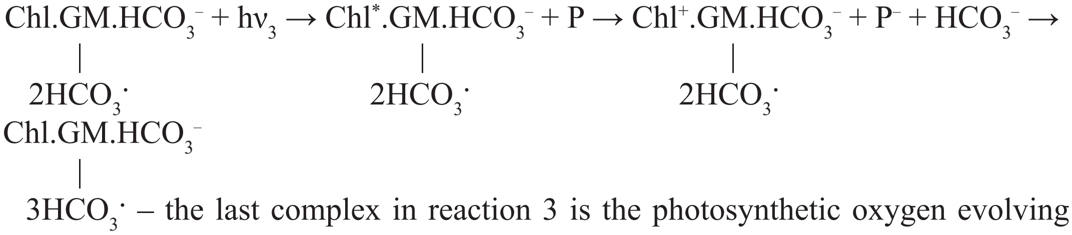
2.



$$\mathrm{Chl.GM.\underset{\overset{|}{HCO_3^{\cdot}}}{HCO_3^-} + h\nu_2 \rightarrow Chl^*.GM.\underset{\overset{|}{HCO_3^{\cdot}}}{HCO_3^-} + P \rightarrow Chl^+.GM.\underset{\overset{|}{HCO_3^{\cdot}}}{HCO_3^-} + P^- + HCO_3^- \rightarrow Chl.GM.\underset{\overset{|}{2HCO_3^{\cdot}}}{HCO_3^-}}$$

– the terminal complex in reaction 2 is the photosynthetic oxygen evolving centre after receiving the second photon. This state reflects the S_2 state in the Kok's model. The effect of the third photon will be described as follows:

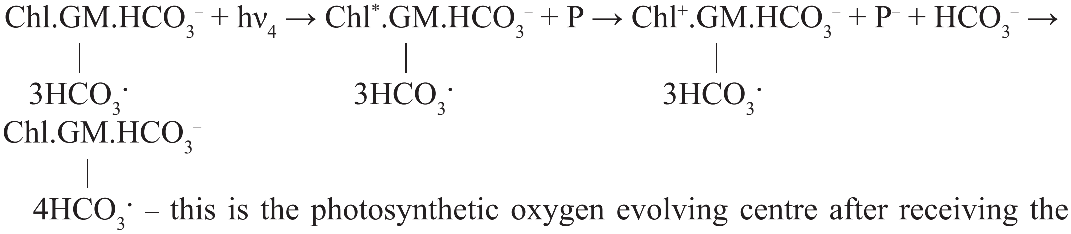
3.



$$\mathrm{Chl.GM.\underset{\overset{|}{2HCO_3^{\cdot}}}{HCO_3^-} + h\nu_3 \rightarrow Chl^*.GM.\underset{\overset{|}{2HCO_3^{\cdot}}}{HCO_3^-} + P \rightarrow Chl^+.GM.\underset{\overset{|}{2HCO_3^{\cdot}}}{HCO_3^-} + P^- + HCO_3^- \rightarrow Chl.GM.\underset{\overset{|}{3HCO_3^{\cdot}}}{HCO_3^-}}$$

– the last complex in reaction 3 is the photosynthetic oxygen evolving centre after receiving the third photon. This state reflects the S_3 state in the Kok's model. And finally the action of the fourth photon will lead to:

4.



$$\mathrm{Chl.GM.\underset{\overset{|}{3HCO_3^{\cdot}}}{HCO_3^-} + h\nu_4 \rightarrow Chl^*.GM.\underset{\overset{|}{3HCO_3^{\cdot}}}{HCO_3^-} + P \rightarrow Chl^+.GM.\underset{\overset{|}{3HCO_3^{\cdot}}}{HCO_3^-} + P^- + HCO_3^- \rightarrow Chl.GM.\underset{\overset{|}{4HCO_3^{\cdot}}}{HCO_3^-}}$$

– this is the photosynthetic oxygen evolving centre after receiving the fourth photon. This state reflects the S_4 state in the Kok's model. The obtained 4 bicarbonate radicals lead to reaction 5:

5. $4HCO_3^{\cdot} \rightarrow 2H_2O + O_2 + 4CO_2$ – oxygen production reaction in photosynthesis and initial CO_2 burst (the first effect of Emerson).

When the concentration of O_2 is increased, the process of blocking the reaction centers starts!

$$\mathrm{Chl.GM.HCO_3^- + O_2 + P + h\nu \rightarrow Chl.GM.O_2 + HCO_3^{\cdot} + P^-}$$

The experimental data show that the number of the remaining effectively working reaction centers is only one working RC to 100 up to 1000 blocked ones!

The essential conclusion from the results presented above, as well as in my previous works (Zeinalov, 2009a, b; 2010), is that in darkness all chlorophyll molecules or their complexes (most probably with glutamate, as proposed by Warburg and Krippahl, 1967), capture HCO_3^- and form the so-called Warburg photolyte – $ChlGMHCO_3^-$.

The presented interpretation can explain the initial oxygen burst (designated by arrow "a" in Fig. 4) in the beginning of the induction period. After incubation for 10–30 min in darkness all blocked ($ChlGMO_2$) in preceding light time centres are transformed in an active state, i.e. in a photolyte form ($Chl.GM.HCO_3^-$). Under irradiation of the system with sufficiently high light the enormous number of the oxygen evolving centres, i.e. all chlorophyll molecules, are in a state to perform charge separation reactions and to evolve O_2, which leads to the extremely fast increase of O_2 concentration, and in turn to the blocking of the essential part of the centres. As was shown, the process of blocking of the photolyte starts for time intervals shorter than 100 ms (Fig. 6). Most likely, the following oscillations in the rate of oxygen evolution ("b", "c" and "d", Fig. 4) are caused by the inhibitory action of the product of the reaction (O_2), i.e. the action of the "negative feedback" regulation.

The mechanism of O_2 production described above should be called uncooperative or Kok's mechanism, i.e. all O_2 producing reactions are performed at a given reaction center or chlorophyll molecule. This mechanism is working essentially in the so-called grana thylakoids regions. The production of O_2 in stroma regions is realized by mutual interaction between O_2 precursors i.e. bicarbonate radicals ($HCO_3^{\cdot}$) produced by different oxygen evolving centers or chlorophyll molecules, that can be considered a cooperative mechanism of O_2 evolution.

DISCUSSION

While the dark reactions of photosynthesis are well described by the Calvin - Benson cycle, the sequence of the so-called light-induced reactions is not clear. The wrongly accepted assumption, i.e. the Concept of Photosynthetic Units, according to which only one chlorophyll molecule among 600 molecules is in state to perform the charge separation photochemical reaction, while the rest are playing only a light harvesting (i.e., a secondary) role, continues to keep our knowledge about these reactions far from reality. Emerson's second mistake (Emerson, 1957) is the postulation of the existence of two different photochemical systems, participating in the light induced electron transport reactions, which was used by Hill and Bendall (1960) for designing the so called Z-scheme. These two wrongly postulated concepts have been accepted by almost all scientists in the area. It is obvious that the interpretation of all experimentally obtained results concerning the light induced reactions of photosynthesis during the last 60-80 years are based on the theoretical consequences arising from these two concepts. As soon as these wrong concepts are rejected, a significant progress in the understanding of the photosynthetic machinery will be achieved.

The debate between Otto Warburg and Robert Emerson, considered as the greatest scandal in all plant physiology, on the maximum quantum efficiency or minimum quantum requirement of photosynthesis, ended without any answer and experimental proof. Even now, so many years after the dispute, no one could give a definite value of these quantities (see Pirt, 1983; Johnson and Barber, 2003). The debate was logically concluded after the postulation of the idea (Emerson, 1957; Emerson and Chalmers 1958) that during the light induced reactions of photosynthesis the electron is submitted consecutively to the action of the two different photochemical systems, i.e. the 4 electrons, needed for the reduction of 1 molecule CO_2 to the level of CH_2O should be transferred by the energy of 8 instead of 4 light quanta. So, after many years of debate, at present we can understand that the real reason for this debate are the experimentally obtained results for the minimum quantum requirement (around 8 – 12 quanta for 1 O_2), accepted as irrefutable results. This value was accepted by Emerson and his adherents and they tried to provide a theoretical co-ordination of this experimental result. Finally, after the discovery of the so-called “Enhancement effect” or “Emerson’s second effect” they postulated the existence of the two different photosystems, and this assumption was used by Hilll and Bendall (1960) for designing the so-called “Z-scheme”. As was shown (see Fig. 9), the mistake of Emerson was the ignoring of the existing initial non-linearity of the photosynthetic light curves that leads to the appearance of this effect, as well as to the increase of the quantum requirement value approximately twice (Zeinalov, 2009b).

On the other hand, Warburg having in view the Einstein law for the photochemical reactions, insisted that this value should not be higher than 4 - 5 quanta per 1 evolved O_2 molecule. However, I have shown earlier (Zeinalov, 2009b) that as a consequence of the non-linearity of the light curves under low light intensity conditions, these low values for the quantum requirement cannot be obtained experimentally. For this reason, Warburg introduced the notion about photolyte. As it was pointed above, Warburg assumed that the photolyte was the complex $ChlGMCO_2$ and all the rest chlorophyll molecules were in a “free state”. So, in his last paper (Warburg and Krippahl, 1967) he estimated the minimum quantum requirement of photosynthesis to be around 3 quanta per 1 O_2 molecule evolved, while the experimentally registered lowest value was around 12 quanta. Unfortunately, at that time the process of blocking of the oxygen evolving centres during the induction time of photosynthesis was not known and the authors could not assume that the “free chlorophyll” in reality was chlorophyll in a blocked state - $ChlGMO_2$.

I have spent more than 45 years of my life in investigating the basic principles of photosynthesis. Nevertheless, I have not found even one observation and experimental piece of data which could be accepted as an irrefutable argument in support of the two generally accepted fundamental hypotheses. During this time many bright and talented scientists have spent much efforts trying to resolve extremely complicated problems created by these two concepts. Many efforts have been made to deal with the structure and organization of the so-called "photosynthetic units" and their different "light-harvesting complexes," so that the two reaction centres of the "two photochemical systems," connected in a series – "Z-scheme", could receive the needed light quanta in a suitable manner. Many problems arising from the two postulated photosystems and their mutual connection and function have been created and much time has been exhausted for solving these artificially created problems. The situation in science of primary light induced reactions of photosynthesis is extremely tragic as a result of the bitter scandal between Warburg and Emerson, confirming the generally accepted principle that "The fruits of the affected state of mind are always bitter". Thus, instead of resolving real existing problems and achieving a real progress in the understanding of the photosynthetic machinery, thousands of old and young scientists continue to lose time and funds, trying to resolve nonexistent problems.

I express my hope that all proofs presented above will be in help to the young scientists of the 21st century. Apparently, they have two alternatives: i) to continue with the interpretation of the newly obtained experimental results using the two imaginary concepts, thus wasting their lives in resolving nonexistent problems or ii) to reject the wrongly introduced dogmas, postulated by Robert Emerson and his adherents (called "Mid-West Gang" by Otto Warburg) by reconsidering in a more careful approach the basic arguments of these two hypotheses, and thus to speed up the understanding of photosynthesis, this unique process maintaining life on Earth. My advice to them is to accept the second option, although I know very well that they will be faced with enormous difficulties. Nevertheless, this is the choice of real science; the alternative is a plain mimicry.

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REFERENCES

Arnon DI, RK Chain, BD McSwain, H Tsujimoto, DB Knaff, 1970. Evidence from chloroplast fragments for three photosynthetic light reactions. Proc Natl Acad Sci USA, 67: 1404–1409.

Arnon DI, HY Tsujimoto, GM-S Tang, 1980. The oxygenic and anoxygenic photosystems of plant photosynthesis: An up-dated concept of light-induced electron and proton transport and photophosphorylation. Photosynth Congress vol II Halkidiki, Greece 7–18.

Emerson R, 1957. Dependence of yield of photosynthesis in long-wave red on wavelength and intensity of supplementary light. Science, 125: 746.

Emerson R, W Arnold, 1932a. A separation of the reactions in photosynthesis by means of intermittent light. J Gen Physiol, 15: 391–420.

Emerson R, W Arnold, 1932b. The photochemical reaction in photosynthesis. J Gen Physiol, 16: 191–205.

Emerson R, CM Lewis, 1939. Factors influencing the efficiency of photosynthesis. Amer J Bot, 26: 808–822.

Emerson R, CM Lewis, 1941a. Carbon dioxide exchange and the measurement of the quantum yield of photosynthesis. Amer J Bot, 28: 789–804.

Emerson R, CM Lewis, 1941b. The quantum efficiency of photosynthesis. Carnegie Inst Yearbook,40: 157–160.

Emerson R, RV Chalmers, C Cedersland, 1957. Some factors influencing the long-wave limit of photosynthesis. Am J Bot, 43: 133–143.

Emerson R, RV Chalmers RV, 1958. Speculations concerning the function and phylogenetic significance of the accessory pigments of algae. Phycol Soc News Bull, 11: 51–56.

Gaffron H, K Wohl, 1936. Zur Theorie der Assimilation. Naturwissenschaften 24: 81–103.

Govindjee, 1963. Emerson enhancement effect and two light reactions in photosynthesis. In: Photosynthetic Mechanisms in Green Plants. Publication 1145, Published by National Academy of Sciences - National Research Council, pp. 318–334.

Govindjee, 2000. Milestones in Photosynthesis Research. In: Younis M, Pathree U and Mohanty P (eds) Probing Photosynthesis: Mechanisms, Regulation and Adaptation. Taylor and Francis, London UK, pp. 9–39.

Hill R, F Bendall, 1960. Function of the two cytochrome components in chloroplasts: a working hypothesis. Nature, 186: 136–137.

Hoganson CW, GT Babcock, 1997. A metalloradical mechanism for the generation of oxygen from water photosynthesis. Science, 277: 1953–1556.

Huzisige H, N Takimoto, 1974. Analysis of Photosystem II using particle II preparation. Role of cytochrome b-559 with different redox potentials and plastocyanine in the photosynthetic electron transport system. Plant Cell Physiol, 15: 1099–1113.

Johnson Z, RT Barber, 2003. The low light reduction in the quantum yield of photosynthesis: potential errors and biases when calculating the maximum quantum yield. Photosynth Res, 75(1): 85–95.

Knaff DB, DI Arnon, 1969. Light-induced oxidation of a chloroplst b-type cytochrome at -196^{o}C. Proc Natl Acad Sci USA, 63: 956–962.

Kok B, B Forbush, M McGloin, 1970. Co-operation of charges in photosynthetic O_2 evolution. I. A linear 4 step mechanism. Photochem Photobiol, 11: 457–475.

McAlister ED, 1939. The chlorophyll-carbon dioxide during photosynthesis. J Gen Physiol, 22: 613–636.

Park RB, PV Sane PV, 1971. Distributionn of function and structure in chloroplast lamelae. Ann Rev Plant Physiol, 22: 395–430.

Pirt SJ, 1983. Maximum photosynthetic efficiency: a problem to be resolved. Biothechnol Bioeng 1, 25(8): 1915–1922.

Raval MK, B Biswal, UC Biswal, 2005. The mystery of oxygen evolution: analysis of structure and function of Photosystem II, the water-plastoquinone oxido-reductase. Photos Res, 85: 267–293.

Renger G, 1997. Mechanistic and structural aspects of photosynthetic water oxidation. Physiol Plant, 100: 828–841.

Tommos C, GT Babcock GT, 1998. Oxygen production in nature. A mettaloradical enzyme process. Acc Chem Res, 31: 18–25.

Vennesland B, 1966. The energy conversion reactions of photosynthesis. In: Krogmann, DW, Powers WH end Biochemical Dimensions of Photosynthesis, pp. 48–61.

Warburg O, G. Krippahl, 1967. Photolyt und Chlorophyll in *Chlorella*. Biochem Z, 346: 418–428.

Zeinalov Y, 1982. Existence of two different ways for oxygen evolution in photosynthesis and photosynthetic unit concept. Photosynthetica, 16: 27–35.

Zeinalov Y, 2002. An Equipment for Investigations of Photosynthetic Oxygen Production Reactions. Bulg J Plant Physiol, 28: 57–67.

Zeinalov Y, 2009a. Is the Concept of Photosynthetic Units Verified. Z Naturforsch, 64c: 459–475.

Zeinalov Y, 2009b. On the minimum quantum requirement of photosynthesis. Z Naturforsch, 64c: 673–679.

Zeinalov Y, 2009c. The Origin of Spectral Effects in Photosynthesis. Z Naturforsch, 64c: 680–686.

Zeinalov Y, 2010. Photosynthesis: Behind the fundamental concepts. Lambert Academic Publishing.