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HISTORY & BIOGRAPHY

LETTER TO THE EDITOR

Hans Kautsky's groundbreaking discovery(ies) in 1931, its scientific environment, and the ensuing developments

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Abstract

In 1931, Hans Kautsky discovered not only chlorophyll (Chl) fluorescence induction ("Kautsky effect") in green leaves but also metastable excited oxygen, now known as singlet oxygen, which he showed to act as an intermediate in dye-sensitized photooxidations of organic substances *in vitro* ("Kautsky mechanism"). While at that time practically nothing was known about the primary reactions of photosynthesis, Kautsky firmly believed that "his" mechanism is also effective in the "Chl-sensitized" conversion of light energy into chemically fixed energy. This erroneous assumption complicated the interpretation of rapid Chl fluorescence induction kinetics, particularly those measured by his student Ulrich Franck in his 1941 dissertation, part of which indicated the existence of two excitonically separated light reactions. This historical note deals with the essence of Kautsky's two discoveries, the scientific environment under which they took place, and the question of why mainstream photosynthesis researchers have largely ignored the ensuing detailed experimental work of Ulrich Franck. The first commented English version of Kautsky and Hirsch (1931) is presented in the Appendix.

Keywords: chlorophyll fluorescence induction; Kautsky effect; Kautsky mechanism of dye-sensitized photooxidations; photosynthesis; singlet oxygen; two photosynthetic light reactions; Ulrich Franck.

Introduction

In 1931, Hans Kautsky (1891–1966) made two outstanding discoveries, one in photosynthesis and one in photochemistry, which have proven of groundbreaking importance in these two fields of science. Thus, Kautsky and Hirsch (1931) were the first to observe light-induced changes of the red chlorophyll (Chl) fluorescence in green leaves that are correlated with photosynthetic activity, a phenomenon which today is well-known as "Chl fluorescence induction" or the "Kautsky effect". In addition, Kautsky and de Bruijn (1931) discovered a metastable form of excited molecular oxygen, later identified as singlet oxygen ($^1O_2$), which they showed to serve as an intermediate in dye-sensitized photooxidations of organic substrates *in vitro*. Among photochemists, the underlying mechanism of such photooxidations to date is referred to as the "Kautsky mechanism". The present historical note primarily deals with Kautsky's discovery of Chl fluorescence induction, which was the starting point for very intensive and broad research on light-induced changes in Chl fluorescence yield as an indicator of photosynthetic activity, lasting until today.

Already in 1941, based on remarkably detailed kinetic information, Kautsky's student Ulrich Franck (1915–1996) postulated in his PhD thesis the existence of "another" (a second) light reaction in photosynthesis, which he

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Dedication: This historical note is dedicated to our friend and colleague Govindjee on the occasion of his 92nd birthday.
Acknowledgments: We thank Govindjee for valuable comments and recommendations. Gert Schansker is thanked for a basic English version of Kautsky and Hirsch (1931) from which the commented and somewhat modified version presented in the Appendix was derived.
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suggested to be responsible for a rapid fluorescence decline in the Chl fluorescence induction kinetics of anaerobic samples. At first glance, Kautsky's discovery of singlet oxygen, $^1O_2$, does not seem to have much to do with the Chl fluorescence induction kinetics. However, the surprisingly strong effect of O_2 removal on the induction kinetics led Kautsky to the conclusion that "his mechanism" is also involved in the primary step of energy conversion in photosynthesis. This conclusion proved erroneous several decades later after spectroscopic methods had become available for unraveling the true mechanism of charge separation in PSII. However, the experimental evidence for "another" (a second) light reaction in photosynthesis has proven fully correct.

This historical note outlines the outstanding contributions of Hans Kautsky and Ulrich Franck to photosynthesis research. In addition to this, we present in the Appendix a first commented English translation of the paper of Kautsky and Hirsch (1931) originally written in German.

Discovery of Chl fluorescence induction kinetics

The introduction of one of Govindjee's most frequently cited papers (with the title Sixty-three years since Kautsky: Chlorophyll *a* fluorescence) (Govindjee 1995) begins with the sentence "*Chlorophyll (Chl) a fluorescence is red and beautiful*…". This description of Chl fluorescence vividly describes how Kautsky and Hirsch must have felt when they first observed "chlorophyll fluorescence induction", *i.e*., complex changes in Chl fluorescence intensity upon a dark–light transition (Kautsky and Hirsch 1931, in the German language), which later became known as the "Kautsky effect". This discovery happened 94 years ago in a darkened room at the University of Heidelberg, Germany, when Kautsky and Hirsch illuminated a dark-adapted leaf with strong UV light. While they took care that the non-absorbed UV reflected in their eyes was negligibly weak, the Chl fluorescence seen by their bare eyes gave them a beautiful red image of the leaf. In addition to the beauty of the deep red color, there were fascinating changes in its intensity, which were interpreted to reflect dynamic changes in the efficiency with which light energy is transformed into chemically fixed energy by the process of photosynthesis. They stated: "*The larger the fraction of the absorbed light energy that is converted into chemical energy, the smaller the fluorescence intensity of the chlorophyll*" (*see* also Appendix, where the English translation of their 1931 paper is presented). The characteristics of the observed time-dependent changes made them realize that the beauty of Chl fluorescence not only pertains to its color but also to its potential to serve as a *pioneering tool* in photosynthesis research.

At that time Germany was a leading country in photosynthesis research, as three Nobel prize winners (Adolph von Baeyer, 1835–1917; Richard Willstätter, 1872–1942; and Otto Warburg, 1883–1970) had been trying to unravel the mechanisms of light-driven O_2 evolution and CO_2 fixation. As early as 1870, Baeyer had speculated that in an early step of photosynthesis, carbonic acid is reduced to the simplest carbohydrate, formaldehyde, which is then "polymerized" to higher carbohydrates. Willstätter and Stoll (1918) presented a model in which CO_2 is first hydrated to carbonic acid, which then is reduced and eventually decomposed into molecular oxygen and formaldehyde by an unspecified, "plant-sensitized" light reaction, with triose and glucose being formed from formaldehyde in a catalyzed dark reaction. Warburg had developed an impressive manometric instrument for time-resolved measurements of O_2 evolution in *Chlorella* and had discovered that continuous O_2 evolution is preceded by an "induction period" lasting several minutes. This showed that "assimilation" (*i.e.*, the photosynthesis process) is much more complex than first thought. While the improved time resolution of manometric measurements for the first time allowed one to analyze the properties of the dark reactions that are involved in O_2 evolution and CO_2 fixation, it was by far too low to also obtain information on the *primary reaction* of "chlorophyll-sensitized energy conversion", the mechanism of which had been much speculated about, but until then had not been accessible to experimentation. Therefore, when Kautsky and Hirsch first observed a similar induction effect in the much more readily measurable Chl fluorescence yield, with changes not only in the minutes but also in the seconds time range, it was clear that their discovery had opened the way for a systematic study of the enigmatic "primary reactions" of photosynthesis.

As mentioned above, in the beginning, Kautsky and Hirsch (1931) just used their bare eyes, which after dark-adaptation of a leaf already revealed such important features as (*1*) an initial low fluorescence yield, followed within a second after onset of illumination by (*2*) a rapid rise to a severalfold higher fluorescence peak (not affected by low temperature or poisoning of photosynthesis) and then within minutes (*3*) a slow decline towards a low steady-state level (slowed down by low temperature and prevented by "poisons") (*see* the English translation of the original paper in the Appendix).

In the following years, Kautsky and coworkers, then working at the University of Leipzig, made remarkable progress in developing new instruments with ever-increasing time resolution and sensitivity for an objective registration of the Chl fluorescence induction curves (for some details and a list of all papers written by Kautsky on Chl fluorescence, *see* the comprehensive review of Lichtenthaler 1992). This work culminated in the dissertation of Ulrich Franck (1941), as summarized in four papers, the publication of which in the "Biochemische Zeitschrift" was delayed and overshadowed by World War II (Kautsky and Franck 1943a,b,c,d). In total, there were 13 closely related communications of Kautsky and coworkers on Chl fluorescence in the Biochemische Zeitschrift, numbered I to XIII.

Ulrich Franck's dissertation and early evidence for two consecutive light reactions in photosynthesis

Ulrich Franck had developed a sophisticated measuring system that allowed reliable detection of the dark-light induction kinetics with 10-ms time resolution, thus opening the way for a detailed analysis of the time-separated rapid

light- and slower dark-reactions, both of which displayed an overwhelming wealth of kinetic information, part of which even now, more than 80 years later, is not fully understood. Ulrich Franck paid particular attention to the phenomenon of a dip phase, termed "die erste Depression" (*i.e.*, "the 1st Depression"), which followed the rapid initial fluorescence rise phase under certain conditions. The dip phase was particularly pronounced after the depletion of the sample of molecular oxygen, whereas the initial rise phase practically disappeared under anaerobiosis. A very careful investigation of the properties of the thus "isolated 1st Depression" revealed that it must be driven by a light reaction, which is energetically separated from the light reaction driving the initial rise. Kautsky and Franck (1943c) concluded that a product of the newly discovered light reaction leads, *via* a temperature-dependent dark reaction, to the regeneration of the "excitation energy acceptors" (*i.e.*, fluorescence quenchers) of the light reaction that drives the initial rise of fluorescence yield. As we know now, the light reaction that drives the initial Chl fluorescence rise corresponds to PSII and the other light reaction that causes the dip (*i.e.*, the delayed quenching of fluorescence *via* the intersystem electron transport chain) is PSI.

More than a quarter century later, after the concept of two consecutive light reactions had been proven and generally accepted (Hill and Bendall 1960, Duysens *et al.* 1961, Duysens and Sweers 1963), the work of Kautsky and Franck was acknowledged and extended by Munday and Govindjee (1969). Unfortunately, however, it has been largely forgotten and is not even mentioned in a recent review on the "evolution" of the concept of two light reactions and two photosystems (Govindjee 2023). Notably, in the evaluation of the contributions of various research groups to this evolution, two different aspects/merits have to be distinguished, namely (*1*) obtaining evidence for two light reactions and (*2*) showing that there are two photosystems. The latter presupposes the concept of "photosynthetic units" consisting of light-collecting antenna pigment systems, with reaction centers where the actual energy conversion takes place. In our opinion, Kautsky and Franck (1943c) were the first to present clear-cut evidence for two light reactions, based on Chl fluorescence induction kinetics, whereas Govindjee *et al.* (1960) were the first to observe that far-red light lowers the quantum yield of Chl fluorescence excited by short wavelength light. With our present knowledge of photosynthesis, the latter observation may be considered the first evidence for the existence of two photosystems, one of which (now known as PSI) lowers the fluorescence yield of the other (now known as PSII) indirectly *via* the electron transport chain. But, at that time Govindjee *et al.* (1960) did not fully understand the observed effect. They just surmised that "the phenomenon may be associated either with a special form of chlorophyll *a* or with an unknown pigment". The full "picture" of the puzzle evolved three years later in the famous study of Duysens and Sweers (1963), in which fluorimetric and spectroscopic measuring techniques were combined (*see* also below).

Fig. 1. Ulrich Franck (1912–1996) in 1969, then Professor of Physical Chemistry at the Rheinisch Westfälischen Technischen Hochschule Aachen and "Doktorvater" of one of the authors (U. Schreiber) (picture provided by Ulrich Schreiber).

Hans Kautsky passed away in 1966, but his research on fluorescence induction (the "Kautsky effect") was carried on by Ulrich Franck and co-workers at the University of Aachen, including one of the authors (U. Schreiber) of this historical note (Franck *et al*. 1969). In 1974, the action spectra of the rapid fluorescence rise and the ensuing fluorescence decline under anaerobic conditions confirmed that these are driven by PSII and PSI, respectively (Schreiber and Vidaver 1974). A photograph of Ulrich Franck taken in 1969 is shown in Fig. 1. After having given a lecture at the University of Aachen in 1969, one of the authors of this historical note (H.K. Lichtenthaler) had the chance to talk to Ulrich Franck in his institute on Chl fluorescence research and photosynthesis. Ulrich Franck was a dedicated scientist, he impressed with his clear scientific concepts and views, and he had a great and kind personality. This discussion was the initiation for H.K. Lichtenthaler to successfully apply Chl fluorescence in his further photosynthesis research.

The discovery of Chl fluorescence induction kinetics and of two photosynthetic light reactions by Hans Kautsky and Ulrich Franck has not only extremely enhanced photosynthesis research. In addition, it was the starting point for manifold applications of the *in vivo* Chl fluorescence in all fields of plant biology not only concerning terrestrial plants and algae but also in limnology and oceanography, such as remote sensing of phytoplankton and terrestrial vegetation. An overview of such applications is found in the book "*Applications of Chlorophyll Fluorescence in Photosynthesis Research, Stress Physiology, Hydrobiology, and Remote Sensing*" (Lichtenthaler 1988), which provides 44 original scientific contributions by various authors.

In retrospect, it appears fair to state that Kautsky and Franck (1943c) discovered the existence of a second

light reaction, which later was called PSI, a discovery which in our opinion has been greatly overlooked in the mainstream literature. The joint work of Kautsky and Franck was brought to a stop by World War II. At the end of the war, Kautsky and co-workers were deported by the US Military Secret Service to the western part of Germany, leaving behind the ruins of their laboratory and equipment. In 1948, they published a summary of Ulrich Franck's dissertation, with some modifications in the interpretation of the data in the light of new information on photosynthesis and Chl fluorescence obtained in other laboratories (Kautsky and Franck 1948a,b). In 1949, Kautsky, eventually, was appointed to the Chair of Silicon Chemistry at the University of Marburg (West Germany), where the focus of his research unavoidably was shifted from Chl fluorescence to silicon chemistry (some more information on his "unorthodox" biography is given below). Nevertheless, he succeeded in setting up a new work group to follow up his and Ulrich Franck's pre-war work on Chl fluorescence, including an analysis of the kinetics of the 1st Depression. But it took more than 10 years until he got around to publishing the outcome of this important work (Kautsky *et al.* 1960). In this paper (also written in the German language), again the existence of two photochemical reactions was postulated. It was explicitly stated that during the process of photosynthesis "*two light reactions succeed one another almost immediately*" ("*zwei Lichtreaktionen folgen fast unmittelbar aufeinander*"). But, just like Kautsky and Franck (1943c), Kautsky *et al.* (1960) did not have the means to show that light absorbed by *two different pigment systems* funnels excitation energy into these light reactions. Nevertheless, Duysens and Sweers (1963) appropriately recognized Kautsky's findings of two consecutive light reactions by clearly stating: "*a recent paper of Kautsky et al. 1960 contains a scheme which is formally similar to ours*". The earlier experimental work of Kautsky and Franck (1943c), however, was not acknowledged.

Ulrich Franck found new employment after World War II at the Max-Planck-Institute of Physical Chemistry in Göttingen, the director of which was Hans Kautsky's friend Karl-Friedrich Bonhoeffer (brother of Dietrich and Klaus Bonhoeffer, both of whom were executed in 1945 for their staunch resistance to the Nazi dictatorship). In these new surroundings, the focus of Ulrich Franck's work was on electrophysiological model systems of nerve pulse excitation and transmission (Franck 1949). But, he also managed to rebuild his Chl fluorescence measuring system, as part of the diploma thesis of his student H. Sundermann, featuring a further improved time resolution of 1.5 ms. In principle, the same measuring system was then reproduced in Kautsky's laboratory in Marburg and used in his late work on Chl fluorescence, as mentioned in Kautsky *et al.* (1960). In 1962, Ulrich Franck was appointed full Professor at the Institute for Physical Chemistry of the Rheinisch Westfälischen Hochschule (RWTH) Aachen (West Germany), where besides several other topics (nerve excitation, chemical oscillations, shock waves, corrosion) he also headed a work group on Chl fluorescence, carrying on and extending the earlier work of Kautsky and Franck (1943a,b,c,d) with improved experimental means and adjusting the interpretation of the obtained data to the newer stand of knowledge on photosynthesis (Franck *et al.* 1969).

Now, 94 years after Kautsky and Hirsch (1931) and 82 years after Kautsky and Franck (1943c), one may ask the question why the discovery of fluorescence induction in 1931 is generally acknowledged and still frequently cited, whereas the discovery of a "second light reaction" (originally described in Ulrich Franck's PhD thesis in 1941) has been widely ignored and forgotten. An obvious possible reason is that the latter discovery happened during World War II when the exchange of scientific information between Germany and other leading countries in photosynthesis research was interrupted. Further, all the papers of Kautsky and Franck were published in German journals (in the German language), to which even after World War II only a few researchers had access (Kautsky and Franck 1943a,b,c,d; Kautsky and Franck 1948a,b). Last but not least, in addition, for other researchers there was a serious *problem in understanding* the content of these papers, not only because of the language but also due to Hans Kautsky's peculiar and "unorthodox" view of the relationship between primary photosynthetic energy conversion and fluorescence yield. To understand this view, another facet of Hans Kautsky's scientific work must be briefly dealt with (*see* below).

"Kautsky mechanism" of dye-sensitized photooxidations

Besides working on Chl fluorescence *in vivo*, the chemist Kautsky was also a pioneer in photochemistry *in vitro*, with a special interest in chemiluminescence in light-driven, dye-sensitized reactions and the role of "metastable oxygen" in those reactions. In 1931, *i.e.*, in the same year of his discovery of Chl fluorescence induction, he carried out an ingenious "three-phase experiment" (Kautsky and de Bruijn 1931), which 75 years later was described as follows by Alexander Greer (a student of Christopher Foote, to whom in the mainstream literature the discovery of singlet oxygen is attributed) (Greer 2006): "*…in 1931 Kautsky and de Bruijn conducted a brilliant series of experiments at the University of Heidelberg. A dye (trypaflavine) and an oxygen-acceptor compound (leucomalachite green) were adsorbed separately on SiO_2 gel beads that were 1.2 and 0.23 mm in size, respectively. These were then mixed and irradiated in the presence of O_2. Oxidation of leucomalachite took place to give malachite green. …The chemistry was found not to be due to diffusion of trypaflavine or leucomalachite, which remained attached to the original beads. The oxygen source was found not to be H_2O. Because trypaflavine and leucomalachite were separated by several millimeters and the compounds were not adsorbed on the same gel granules, Kautsky's "three-phase test" suggested the formation of a diffusible O_2 species, assumed to be in the $^1\Sigma_g^+$ state. Kautsky was challenged almost immediately about his mechanistic interpretation involving $^1O_2$*".

To cut a long story short, 30 years of controversy followed, before Kautsky's original finding that $^1O_2$ acts as an intermediate in the photooxidation of organic substrates (Kautsky and de Bruijn 1931) eventually was confirmed by Christopher Foote (Foote and Wexler 1964). Thereafter among the photochemists, the above-mentioned mechanism has become known as the "Kautsky-mechanism".

Kautsky's error in assuming that "his" mechanism applies to primary energy conversion in photosynthesis as well

In the context of the present historical note, it may be considered almost "tragical" that Hans Kautsky firmly believed that "his" mechanism not only functions *in vitro* but also in the *in vivo* transformation of excitation energy into chemically fixed energy in the primary reaction of photosynthesis. In a review of his pioneering *in vitro* experiments on singlet oxygen, which he called "excited metastable oxygen" he wrote (Kautsky 1939): "*I should also like to mention shortly my observations on the numerous regular changes with time of the intensity of chlorophyll fluorescence in green plants, and the dependence of these changes upon various internal and external factors. Free and bound oxygen is most probably the cause of the decrease of fluorescence with time which occurs in the green particles of the chloroplasts (grana). By the transfer to these compounds, the excitation energy of the chlorophyll may be stabilized in the form of chemical energy, and become concentrated and available at appropriate places in the assimilatory apparatus*".

At the present state of knowledge, it is clear that Kautsky made a serious mistake when he assumed that "his" *in vitro* mechanism of dye-sensitized photooxidation of organic substrates plays a role in the *in vivo* Chl-sensitized primary reaction of photosynthesis as well. This mistake, however, is quite understandable given the exceptional changes in Chl fluorescence induction caused by O_2 removal (Kautsky and Hormuth 1937, Kautsky and Franck 1943c). As was shown much later, O_2 removal in green algae indeed leads to *complete inhibition of energy conversion in PSII*, as reflected by maximal fluorescence yield at the start of a dark-light transition (Schreiber and Vidaver 1974, 1975). It turned out, however, that by O_2 removal from the living cells, complex changes in cell metabolism are induced that eventually lead to the reduction of the intersystem electron transport chain, including the primary acceptors of PSII. The latter become reoxidized by PSI *via* the intersystem electron transport chain. Hence, differently from its role in dye-sensitized photo-oxidation *in vitro*, O_2 is *not* involved in the primary reactions of photosynthesis, which consist of light-induced redox reactions (charge separation across the thylakoid membrane).

Hans Kautsky's unique and unorthodox biography as an artist and scientist

For understanding Hans Kautsky's unique, although somewhat "unorthodox" way of photochemical research, a look at his recently published biography is revealing (Behrends and Beyer 2023). In his Curriculum Vitae (written before his appointment to the University of Marburg in 1945), he wrote about himself: "*Until 1915 I lived in Vienna. My first chemistry lesson in 1906 at the Oberrealschule was a decisive school experience. I set up a small laboratory in the cellar of our house, where 6 years later I undertook independently my first major chemical work and succeeded in advancing into a new field of inorganic chemistry (siloxene). This work became the impetus for my university studies, which I began in Berlin in 1915. There my father was Kgl. Preuss. Hoftheatermaler*". Hans Kautsky's father and grandfather were internationally renowned painters and he, also, first intended to follow in their footsteps: "*Originally, I didn't think about studying at university, because I had chosen painting as my real profession and my father was very supportive of this inclination by letting me attend painting courses and work with talented artists at home and abroad. This took me to France, Holland, Belgium, Italy, and Switzerland. There is nothing like traveling to broaden one's mental horizons and gain a freer human judgment*".

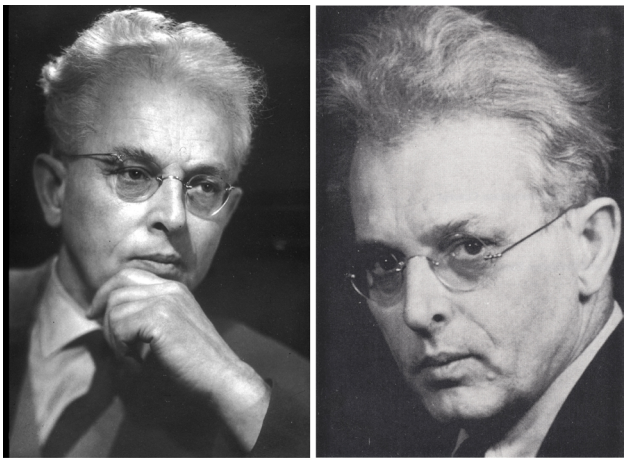

Fig. 2. Two photographs of Hans Kautsky (1891–1966) taken during his time as Professor of Silicon Chemistry at the University of Marburg, Germany, in 1949–1959 (provided by H.K. Lichtenthaler).

Hans Kautsky, the former artist painter (for a summary of his life *see* Lichtenthaler 1992, pages 50 and 51), remained an artist throughout his whole life, which unavoidably also influenced his way of experimentation and scientific observation. Two photos (Fig. 2), taken at different times of his life, show Hans Kautsky as a determined personality. He was fascinated by colors, both in nature and in the darkened rooms where he studied chemiluminescence and chlorophyll fluorescence. His scientific approach was perfectly described by one of his students and coworkers in Marburg, the chemist Gerhard Fritz (Fritz 1981), then Professor of Inorganic Chemistry at the University of Karlsruhe and a close colleague of one of the authors (H.K.L.) of this historical note: "*It is probably almost unprecedented that the chemical experiments of a high school student formed the basis of scientific work that was not properly understood for a long time and still*

presents us with numerous unresolved problems today. Kautsky never pursued questions that were "modern" within inorganic chemistry. He was self-taught and completely unorthodox in his approach to problems. This put him in an outsider position, which is the only reason why he did not receive the recognition he deserved in his academic career".

Indeed, in the case of his very first discovery, a new class of oxygenated silicon compounds, the "siloxenes", Hans Kautsky was far ahead of his time. His first publication (Kautsky 1921), in which the synthesis of these structurally unique, sheet-forming compounds is described, is still cited (*see e.g.*, Ryan *et al.* 2020). They are now generally referred to as "Kautsky siloxenes" and play a role in the development of new types of semiconductors based on "silicon nanosheets".

Returning once more to the question of the reasons underlying the drastically different acceptance of Kautsky and Hirsch (1931) and the much more detailed work of Kautsky and Franck (1943a,b,c,d; 1948a,b) one may conclude that "simplicity beats complexity" or in other words: Kautsky and Hirsch (1931) managed to describe a fundamental natural process in a less than one-page report, presenting just one simply structured figure. The essential content of this 1931 paper, very clearly summarized in a single figure, is easy to understand, even by readers who do not understand German. It is probably fair to assume that when this paper is cited today, the authors have not read the original paper themselves but have only relied on what has been written about it in other frequently cited papers. Since no English translation of Kautsky and Hirsch (1931) is available, we present an English version of this groundbreaking publication in the Appendix below.

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Appendix. Translation from German into English of Kautsky and Hirsch (1931)

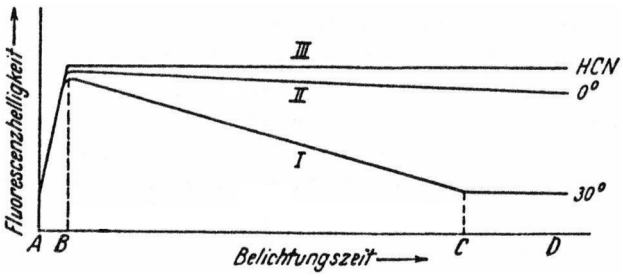
This historically important paper was published in the form of a "Kurze Originalmitteilung" (Short original communication) in Die Naturwissenschaften 1931, Volume 19, issue 48, p. 964. This particular kind of publication had to be limited to a maximum of one printed journal page.

The following translated text provides in [*square brackets*] some short explanations that may facilitate understanding, as well as some more extended Comments that are attached at the end of the translated text.

New experiments on carbonic acid assimilation [*i.e., on photosynthetic CO_2 fixation, see Comment A for some information on the mainstream understanding of photosynthesis in 1931*].

We illuminate leaves of various origins and observe upon onset of illumination[1)] the consecutive temporal changes of the intensity of chlorophyll fluorescence [*the deep red Chl fluorescence was viewed by bare eyes, presumably using a red glass filter for protection, although this is not explicitly stated*]. Through this direct way of seeing, one can observe the extremely revealing, structured prelude to assimilation. Our observations justify the assumption that the larger the fraction of the absorbed light energy that is converted into chemical energy, the lower the fluorescence intensity of the chlorophyll. This indicates that the chlorophyll within leaves, except for its association with carbonic acid, is energetically well isolated [*this means that the putative Chl carbonic acid complex is the only quencher of excitation energy*].

The observations made so far are schematically summarized in the following figure by three curves.



Schematic illustration of the changes in the fluorescence intensity of leaves, observed immediately after the onset of illumination, at 30°C (*I*), 0°C (*II*), and after poisoning with cyanide (*III*). *Abscissa:* Irradiation time (Belichtungszeit). *Ordinate*: Fluorescence intensity (Fluorescenzhelligkeit).

Curve *I* [*measured at 30°C*] is subdivided into 3 phases over time: (*A–B*): a very fast increase, from low fluorescence to maximal intensity; (*B–C*): a slow decline from the peak to a low fluorescence level; (*C–D*): *i.e.*, from *C* until the end of the experiment, a constant low fluorescence level. Curve *II* shows the changes in fluorescence intensity at 0°C, and curve *III* shows the changes after poisoning with hydrogen cyanide.

(*C–D*) corresponds to the state with a constant normal rate of CO_2 fixation under the chosen conditions, which is reached several minutes after the start of illumination only. At a given high rate of energy conversion [*i.e., from excitation energy into chemical energy*], the fluorescence is very low [*this, of course, is true for curve I only*].

The *B–C* part of the curve can be equated with the induction time of assimilation measured by O. Warburg[2)], also coinciding well with it in time [*Warburg measured assimilation manometrically via the amount of evolved oxygen*]. Typical for this induction phase (*B–C*) is the

strong temperature dependence (curve *II*) and the complete disappearance of the fluorescence decline (*i.e*., of the equivalent, gradual increase of the rate of assimilation) that is caused by inhibition with cyanide (curve *III*). Hence, during phase (*B*–*C*) a typical chemical, catalytic reaction occurs (peroxide decomposition, Blackman reaction) [*this sentence calls for some explanation given in Comment B*]. Particularly remarkable is the fact that the assimilation enzyme becomes active only gradually during the illumination. At the maximum intensity *B* of the curve *I*, its activity [*i.e., of the enzyme*] is not zero, but very low. After darkening at time point *C*, it takes, similarly to the induction phase, many minutes before the initial dark state of the leaves is reached again, *i.e*., that the decline of the fluorescence intensity induced by a second illumination is of the same length and amplitude as the first time.

(*A*–*B*) is the primary, purely photochemical reaction. Poisoning with cyanide and changes in temperature are not effective during this phase. The already at low light intensities remarkably rapid increase of fluorescence intensity to its peak at *B* reflects the establishment of a light equilibrium: chlorophyll–carbonic acid + hυ ↔ chlorophyll–formaldehyde peroxide [*Comment C*]. This peroxide compound is relatively stable and does fluoresce. In the case of leaves poisoned with cyanide, just the light-induced equilibrium is established: maximal [*fluorescence*] intensity remains undiminished even over long periods of time. Under these conditions, the leaf does not assimilate carbonic acid. A similar situation is given at 0°C. If the leaves are suddenly darkened at the fluorescence maximum B (*see* curves *I*, *II*, and *III*), the peroxide complex is completely decomposed within seconds: when after such short dark periods illumination is repeated, the same increase of fluorescence intensity is observed again.

The here attempted interpretation of the new observations agrees more or less with the ideas of R. Willstätter[3] on the process of assimilation.

Based on the newly gained insights, detailed further investigations of the assimilation problem at the physiological as well as purely synthetical levels are planned [*It may be assumed that with "synthetical level" Kautsky had his experiments on dye-sensitized photooxidations of organic substances in mind. Notably, the manuscript of Kautsky and de Bruijn (1931) describing the role of "metastable oxygen", now known as singlet oxygen, was submitted just one month later*].

Heidelberg, Chemisches Institut der Universität,
19th October 1931.

H. Kautsky and A. Hirsch

[1] Ultraviolet light (*e.g*., of an analytical quartz lamp) and of the visible spectrum only the blue light serve equally well to induce these fluorescence responses, provided the intensities are more or less equal [*When using "white light" as an excitation source, Kautsky and his coworkers applied a blue ammonia copper sulfate solution to ensure that mainly violet and blue radiation excited the red chlorophyll fluorescence*].

[2] O. Warburg. – Biochem. Z. 103, 188 (1920).

[3] R. Willstätter und A. Stoll. Untersuchungen über die Assimilation der Kohlensäure [*Investigations on the assimilation of carbonic acid*]. – Berlin: Julius Springer 1918.

Comment A

The German word "Kohlensäure" means "carbonic acid" in English. When Kautsky and Hirsch speak of "assimilation", they mean the process of photosynthetic fixation of CO_2, which in 1931 still was poorly understood. It was known that for every fixed CO_2 molecule one molecule of O_2 is evolved, that somehow water must be involved, and that eventually glucose is synthesized. However, the actual mechanisms were largely a matter of speculation, which given the presently known complexity of the overall process is not surprising. Practically nothing was known about primary photochemistry, *i.e*., the mechanism by which the light energy absorbed by chlorophyll is transformed into chemically fixed energy.

In 1931, the understanding of photosynthesis at least in Germany was dominated by the views of Richard Willstätter and Otto Warburg, partially based on older ideas of Adolph von Baeyer, all of them renowned Nobel prize winners. The prevailing view was that chlorophyll forms a complex with carbonic acid, that upon illumination the latter is somehow reduced to a "formaldehyde peroxide" and that O_2 is evolved when the peroxide-complex is "decomposed" in an enzyme-catalyzed reaction. The idea was that after the decomposition 3 formaldehyde molecules would form a triose or 6 formaldehyde molecules would combine to produce a glucose molecule. This and other early ideas on the mechanism of photosynthetic CO_2 fixation were nicely outlined by Eugene Rabinowitch in his impressive (and voluminous) treatise on Photosynthesis (Rabinowitch 1945) (*see e.g*., Chapter 3, pp. 51–56 and Chapter 8, p. 172). From our present knowledge of photosynthetic CO_2 assimilation, *i.e*., the well-known Calvin–Benson cycle, these early ideas may appear quite confusing and they probably were not clear to Kautsky as well. But, being a newcomer in photosynthesis, in his first communication on this topic he was well advised to try interpreting his novel kind of experimental information using the language and concepts of the leading scientists of his time. As we know, this changed in his later work, when he disproved the participation of carbonic acid in the primary processes and instead postulated a pivotal role of oxygen.

Comment B

The term "Blackman reaction" calls for some explanation. Kautsky used it in the sense of an "enzyme-controlled dark reaction" to be distinguished from the light-controlled "primary reaction". About 1905, Blackman started investigating the "limiting factors" of photosynthesis by measuring light saturation curves of the O_2 evolution rate in dependence on CO_2 concentration and temperature. The observed increase of maximal rate by increased temperature under light- and CO_2-saturated conditions

proved the existence of an enzyme-controlled dark reaction that limits the rate of photosynthesis at elevated light intensities. Later the term "Blackman reaction" has been generally used synonymously with the temperature-dependent dark reaction(s) of photosynthesis, which determine the "ceiling" of light-response curves.

Comment C

As already outlined in Comment A, Kautsky followed the mainstream understanding of his time that carbonic acid forms a complex with chlorophyll and that the carbonic acid in this complex is photo-reduced to formaldehyde peroxide. Although not explicitly stated, the reductant in this reaction had to be water, as explained by Rabinowitch (1945) (*see* Chapter 3, p. 52). Kautsky considered the putative Chl–carbonic acid complex a quencher of Chl fluorescence. From the rapid initial increase of fluorescence (part *A–B*), he had to conclude that this quencher is rapidly exhausted upon illumination after dark adaptation. Presumably, the formation of a "light-equilibrium" at the fluorescence peak *B* is postulated, because when the light is switched off at *B*, the induction of the initial rise can be reproduced already after a couple of seconds. This is in contrast to the much slower response when the light is switched off in the high quenching state at the end of the curve (part *C–D*). In this case, it takes several minutes, until upon light-on a high fluorescence intensity can be induced again. The slow decline towards a high quenching state (part *B–C*) is interpreted in terms of a gradually accelerated stimulation of the rate, with which after decomposition of the Chl–formaldehyde peroxide complex the quencher (*i.e.*, the Chl–carbonic acid complex) is regenerated. In the control leaf at 30°C, the final quenching effective in the *C–D* part of the induction curve is close to equal to that at the very beginning of illumination (at time point *A*). This means that in a fully "light-activated" state, the regeneration of quenchers is sufficiently fast to ensure that at the given light intensity the dark reactions are not limiting.