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The study of reaction centers and of the primary and associated reactions of photosynthesis by means of absorption difference spectrophotometry: a commentary by

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on 'Reversible changes in bacteriochlorophyll in purple bacteria upon illumination'
by L.N.M. Duysens, W.J. Huiskamp, J.J. Vos and J.M. van der Hart
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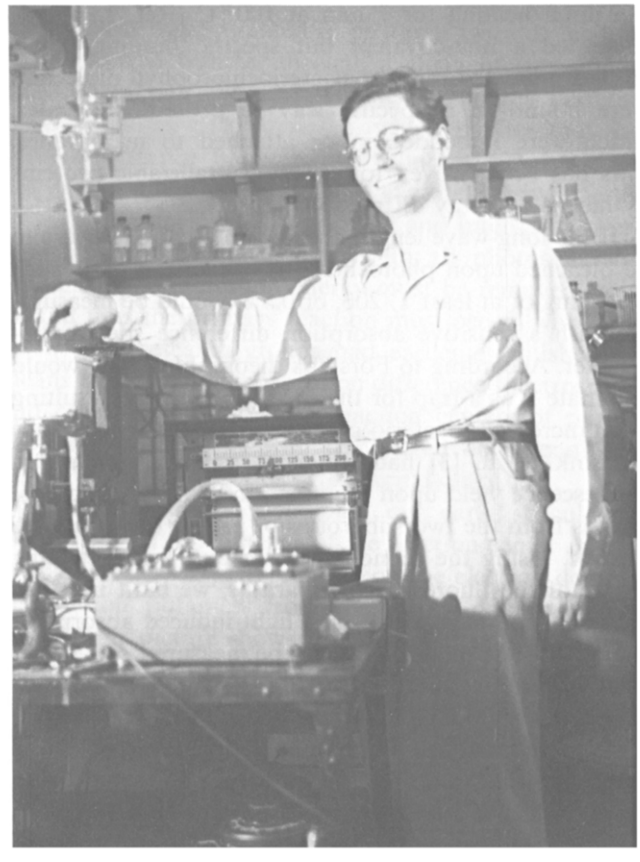
The history is discussed of the introduction of absorption difference spectrophotometry in photosynthetic research and its role in the discovery of reaction centers and of primary and associated reactions.

Before 1947, nothing was known about photosynthetic intermediates and their reactions with the exception of largely incorrect or weakly supported speculations. A few decades later, a large part of this unknown area had been charted, due mainly to the introduction of new methods. One of these methods, sensitive absorption difference spectroscopy, was applied for the experiments described in the reprinted article, in which the discovery is reported of the primary reaction of bacterial photosynthesis. Analogous primary reactions were subsequently discovered in all other photosynthetic organisms.



Louis N.M. Duysens (taken in 1953 at the department of plant biology, Carnegie Institution, Stanford, CA, measuring an absorption difference spectrum).

Ideas leading to the search for a reaction center. The introduction of absorption difference spectroscopy in photosynthesis

The reprinted article was based on ideas and experiments presented in my doctoral thesis [1], and cannot be fully understood without this background. I had concluded from action spectra of (bacterio)chlorophyll fluorescence and photosynthesis, that light energy absorbed by various pigments was transferred to photosynthesis only via (bacterio)chlorophyll. Assuming that the pigments were randomly distributed, I estimated, using Förster's theory of inductive resonance [2], that the average number of transfers of excitation energy between (bacterio)chlorophyll molecules was roughly 750, and concluded (Ref. 1, p. 90) that "transfer of energy with high efficiency can occur from chlorophyll to reaction centers present in a concentration 1/200 of that of chlorophyll". The theory of transfer required that the energy trapping molecule in the reaction center must be a substance with an absorption band at the same or slightly longer wavelength than that of the longest wavelength band of (bacterio)chlorophyll. I

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called this hypothetical trapping molecule P, an abbreviation of pigment. The bulk of the bacteriochlorophyll, the energy transferring bacteriochlorophyll, later to be known as antenna bacteriochlorophyll, was assumed to be photochemically inactive. Since the concentration and thus the contribution of P to the absorption spectrum was considered to be small, the normal absorption spectra (broken lines in Fig. 2) would be due largely to antenna absorption. I assumed that in *Rhodospirillum rubrum* (left-hand side) only one type of antenna bacteriochlorophyll occurred, which I called B890. The absorption band at 800 nm was erroneously attributed to a minor band of the B890 molecule (see below). The hump and maxima in the spectrum of *Chromatium vinosum* (right-hand side) at 890, 850 and 800 nm, were caused by different 'types' of bacteriochlorophyll, called B890, B850 and B800. The fact that only one kind of bacteriochlorophyll had been found in extracts with organic solvents, and the occurrence of shifts of absorption bands from 890 nm to 800 and 850 nm upon heating for 1 min at 100 °C (Ref. 1, p. 30), suggested a non-covalent but specific binding. Later studies indicated that the bacteriochlorophyll molecules were bound in a specific way to protein molecules, which were imbedded in or attached to membranes. This has been proven for a few crystallizable proteins by means of X-ray diffraction.

If the long-wave length absorption band of P were to be bleached upon photochemical conversion of P, such bleaching of at least 1/200, or 0.5%, would be measurable with a sensitive absorption difference spectrophotometer. According to Förster's theory, bleaching would eliminate P as a trap for the excitation energy, resulting in an increased (bacterio)chlorophyll fluorescence. Since Wassink et al. [3] had observed a large increase in fluorescence yield upon illumination of purple bacteria, species from the two subgroups of purple bacteria were studied. Using the monochromator and light-detecting device of the fluorescence apparatus, we built in a few days an apparatus with which light-induced absorption changes of less than 0.1% could be measured. We found what we were looking for (see Fig. 2): the disappearance of an absorption band at the location of the longest wavelength band at 890 nm of bacteriochlorophyll. Note that a positive value for the difference spectrum (solid curve) indicates a *decrease* in absorption upon illumination, that the scale of this spectrum is expanded 10-times for the left-hand figure and that the absorption changes do not further increase with increasing intensity. This appeared to indicate that P existed, that P was presumably a form of bacteriochlorophyll and was present at a concentration 3% of that of B890, a concentration sufficiently high to allow P to act as a trap of more than 90% efficiency.

Discussing the reaction center model in my thesis (Ref. 1, p. 69), I remarked: "The optimal change in absorption will be obtained under conditions, which favor the accumulation of the bleached form of pigment P, (which) may be enhanced by the lack of substrates of photosynthesis. The exhaustion of the hydrogen donor in a suspension of *Chromatium* causes a strong increase in fluorescence yield [3]). In measurements, to be discussed elsewhere, it was found indeed, that the change in absorption was generally far stronger in a substrate-free medium than in a medium to which substrate had been added". These measurements are shown here in Fig. 1.

Since theory fitted nicely with experiment and was consistent with other experiments in the literature, the conclusion would have been that the reaction center model with P as trap was correct, but difficulties in the explanation of the decrease in absorption at 810 nm in *Rhodospirillum* threw doubt on this conclusion. When these difficulties were resolved in 1955, we could interpret all experiments using the trapping model: the absorption decrease around 890 nm shown by the solid curves in Fig. 2 was caused by the bleaching of the trap P and the absorption changes between 750 and 830 nm by absorption changes in one or a few pigment molecules with absorption bands possibly similar to that of B800 in *Chromatium*.

Being busy with other things which did not permit delay, I hastily wrote the article, omitting even the discussion of Fig. 1, perhaps too optimistically assuming that most interested readers would be aware of the discussion in my thesis, which I had sent to practically all senior people in the field.

The primary reaction and cytochrome oxidation in purple bacteria

After obtaining the Ph.D. degree, I continued research in photosynthesis in the U.S.A. in the laboratories of C.S. French in 1953 and of E.I. Rabinowitch in 1954, using absorption difference spectrometers, which I constructed with locally available parts such as monochromators and recorders. In contrast with the research described in the preceding section, these experiments were not based on any model, but on the simple idea that some photosynthetic intermediates would be colored redox components, which would change their absorption spectrum when changing in redox state upon illumination. I just searched different spectral regions in various photosynthetic organisms for light-induced absorption changes. The light-induced difference spectrum of *Rhodospirillum* in the visible region appeared, to my surprise, to be similar to the difference spectrum of a respiratory intermediate, cytochrome *c*. These experiments showed that a cytochrome was efficiently oxidized upon illumination. Since the light-induced bleaching of P occurred more readily in the presence of air and in the absence of a hydrogen donor, this suggested that the

bleaching of P was a photooxidation. The last equation of the reprinted article states that cytochrome is oxidized by photooxidized bacteriochlorophyll (the P of the reaction center). The oxidized cytochrome was postulated to oxidize in its turn a so-called hydrogen donor [4]. In oxygenic photosynthetic organisms the hydrogen donor is water, but in purple bacteria it is an organic substrate or an inorganic one, such as hydrogen sulfide. The majority of scientists accepted at that time van Niel's hypothesis [5] that in all photosynthesizing organisms the primary reaction was the splitting of water into H and OH, which were both bound to enzymes. H was used via intermediates for the reduction of carbon dioxide. In purple bacteria, OH oxidized the hydrogen donor, in oxygenic organisms 4 OH recombined to oxygen and two water molecules. In the electron transfer chain, indicated by our experiments:

$$\text{H-donor} \rightarrow \text{cytochrome} \rightarrow \text{P}^* \rightarrow \text{X} \rightarrow \text{CO}_2 \qquad (1)$$

the water splitting hypothesis is superfluous. Now, most people do not know that van Niel ever proposed this hypothesis, which was very popular for some time. In the simplified scheme the arrows represent electron or hydrogen transfer and the asterisk indicates that the electron moves from the excited state of P to X, resulting in oxidized P and reduced X. X is an unknown intermediate. This scheme is based on an earlier and fruitful idea of van Niel [6] that photosynthesis essentially amounts to the transport of hydrogen from the hydrogen-donor to carbon dioxide.

Very soon after my demonstration that significant reversible light-induced absorption difference spectra and kinetics could be measured in suspensions of photosynthesizing cells, in various laboratories absorption difference spectrophotometers were being constructed for research in photosynthesis. At a conference on photosynthesis (see Ref. 7) held at Gatlinburg, Tennessee, in October 1955, the very month the reprinted article was submitted, there were already contributions from the laboratories of B. Chance, C.S. French, E.I. Rabinowitch and H.T. Witt, based mainly on experimental results obtained by means of absorption difference spectrophotometry.

The discovery by A.W. Frenkel of cyclic phosphorylation in purple bacteria and the observation by Chance and co-workers and by myself that two or more cytochromes were photooxidized, led to the proposal of a scheme (Duysens, Ref. 7, pp. 164–173) containing an additional 'cyclic' chain, transferring electrons between oxidized P and reduced X via another cytochrome, responsible for the generation of ATP, which was needed for the reduction of carbon dioxide by reduced X.

The reaction center in purple bacteria

By exposing closed photosynthetically active membrane fragments, called chromatophores, to 0.01 M ferricyanide for 1 min, a bleaching occurred in the dark at 880 nm, which was reversed within a few seconds by adding 0.023 M ferrocyanide. The redox potential for half bleaching was between 0.40 and 0.47 V [8], which is more oxidizing than the midpoint potentials of the bacterial cytochromes. The spectrum of this bleaching was similar to that produced by illumination [9]. Another piece of evidence strongly supporting the reaction center model was the observation [10] that the fluorescence yield, f, of suspensions of *Rhodospirillum rubrum*, under a range of conditions causing large variations in this yield, depended only upon the concentration of oxidized P, P_{ox}.

Direct evidence for cytochrome oxidation by oxidized P was first obtained by Parson [11]. He showed by absorption difference spectroscopy, using a laser flash as a light source, that, in certain species of purple bacteria in which a cytochrome was extremely rapidly photooxidized, P was bleached within a fraction of a microsecond and recovered in the dark in a few microseconds with the same kinetics with which the cytochrome was oxidized.

Clayton observed that in an old culture of a mutant of a purple bacterium the antenna pigments had been largely and irreversibly bleached, but that light-induced absorption changes spectrally similar to and thus probably caused by the reaction center still occurred (Fig. 5, p. 206, of Ref. 12). Upon illumination, the remaining long-wavelength band, in his preparations located at 870 nm, was reversibly bleached to an extent of about 50%. If it is assumed that the remaining 50% absorption is due to a trace of B870, this is consistent with the conclusion that the long-wavelength absorption of P is completely bleached, because of the strong increase in fluorescence yield upon P bleaching [10]. In Clayton's preparation a second, narrower but larger band at 800 nm was shifted to slightly shorter wavelength. This confirmed an earlier suggestion (Ref. 7, p. 168), that bleaching of P890 caused a shift of an 800 nm absorption band, later shown to be due to two bacteriochlorophyll molecules, B, in the reaction center. Reed and Clayton [13] succeeded in isolating a reaction center preparation by detergent extraction followed by sucrose density centrifugation, and Reed and Peters [14] found that the reaction center complex contained 4 bacteriochlorophyll molecules and 2 bacteriopheophytin molecules; bacteriopheophytin is bacteriochlorophyll without the central magnesium atom.

The study of this complex and similar complexes of various bacteria, both isolated and in the intact membrane, was continued in a large number of laboratories with application of all practically all physical techniques

available. I can mention only a few of the main results. Mainly by means of electron spin resonance and associated techniques, it was established that P is a bacteriochlorophyll dimer and that oxidized P is the Γ-cation radical of this dimer. The primary reaction is the transfer of an electron from P, possibly via one of the two remaining bacteriochlorophyll molecules, to a pheophytin molecule, Ph, in about 5 ps (a picosecond equals 10^{-12} s). Then the electron is transferred in 200 ps to a quinone molecule, also bound to the reaction center. The time courses were again measured with absorption spectroscopy, using a picosecond laser as source for generating actinic and measuring pulses. The reaction center is a protein, traversing the membrane. P and the quinone occur at opposite sides of the membrane, and, in bacteria with a fast, microsecond, cytochrome oxidation, a cytochrome is attached to the P-side of the reaction center. These and a number of other conclusions concerning the location and orientation of the electron transferring conjugated systems were confirmed when the reaction center of the purple bacterium *Rhodopseudomonas viridis*, of a molecular mass of 150 kDa, was crystallized and its structure was determined by X-ray crystallography [15], an achievement awarded with the Nobel Prize, 1988, for Chemistry to H. Michel, crystallography, R. Huber, director of the Max-Planck Institute for Biochemistry at Martinsried near Munich, where the research was carried out, and J. Deisenhofer, X-ray diffraction.

The discovery of reaction centers in oxygenic organisms

The reaction center of system 1

In 1954 and 1955 I also measured light-induced absorption difference spectra in various algae. In all species cytochrome, oxidation occurred, but I did not find an appreciable light-induced bleaching of an absorption band at or slightly beyond the long-wavelength chlorophyll *a* maximum at 680 nm, caused by a trap analogous to P in bacteria. A high rate constant for dark reduction of oxidized P might minimize its accumulation at moderate actinic intensities. Later, Bessel Kok, working at the agricultural 'university' at Wageningen, had constructed an absorption difference spectrophotometer allowing appreciably higher actinic intensities than did my apparatus. He discovered [16] in species of all groups of oxygenic organisms studied, a light-induced absorption decrease at 705 nm. This wavelength and also the order of magnitude of the changes was as to be expected for a trap like the bacterial P. Kok concluded, "The location of the red absorption maximum of this hypothetical pigment might suggest that it acts as the final light trap in photosynthesis". I think he confined himself to such a weak statement because he did not understand the kinetics of the absorption changes. When these were understood better, Kok and Hoch [17] called the trap P700, presumably to distinguish it from the bacterial trap, which was then called P890 or P870. Also P700's midpoint potential, 0.46 V, determined in a similar way to that of P890, was about the same as that of the bacterial trap.

The reason for the complicated kinetics of P700 was that the light-induced photooxidation of P700 was opposed by a photoreduction due to a second light reaction driven by a different pigment system from that causing oxidation of P700. Both pigment systems contained chlorophyll *a*. Kok and Hoch thought, however, that oxygenic organisms contained only one reaction center, P700, and that oxidized P700, after being transformed by dark reactions, was reduced upon receiving electronic excitation from the second pigment system.

Unaware of Kok's experiments, we had been studying the reactions of a cytochrome in the red alga, *Porphyridium cruentum*, and had found that cytochrome oxidized by light of 680 nm, absorbed mainly by chlorophyll *a*, which was later called light 1, was reduced again by superimposing light of 560 nm, light 2, absorbed mainly by an accessory pigment, phycoerythrin [18]. It was known that chlorophyll *a* was present in two spatially separate forms, a fluorescent form, to which excitation energy, absorbed by phycoerythrin was transferred, and a predominant non- or weakly fluorescing form [1]. Since the cytochrome oxidation, driven by light 1, and its reduction, driven by light 2, occurred with a reasonably high efficiency, we concluded that two spatially separated photosynthetic reactions were present. One reaction was driven by light 1, absorbed by 'system 1' containing the weakly fluorescent chlorophyll *a*. This reaction, like the photoreaction in purple bacteria, oxidized a cytochrome and produced the reducing equivalents for carbon dioxide reduction. Another spatially separate reaction, which was driven by light 2, absorbed by system 2, containing the fluorescent chlorophyll *a* and a major fraction of phycoerythin, reduced the cytochrome and produced the oxidizing equivalents for oxygen production. Kok's P700 fitted nicely into this scheme as the reaction center trap of system 1. A historic account of the development of ideas and research leading to the discovery of the two photochemical systems is to be published [19].

The reaction center of system 2

When in 1962, for a number of species, various properties of the two photochemical systems had been established, I thought the time was ripe for a study of the fluorescence kinetics, because many hitherto unexplained fluorescence phenomena might be due to effects of the two photosystems.

It took only a very short time to show that alternating actinic light 2 and 1 rapidly increased and decreased the chlorophyll *a* fluorescence yield. This could simply be explained by the assumption that a redox inter-

mediate, called Q, in the electron transfer chain between the two systems quenched the chlorophyll a_2 fluorescence in the oxidized but not in the reduced state [20]. Although, in short-timescale experiments, fluorescence yield and reduction state of cytochrome *f* moved roughly parallel in alternating light 1 and 2, the hypothetical redox intermediate was different from cytochrome *f*, because in the dark (or in weak measuring light) the cytochrome *f* was in the reduced state, but the fluorescence yield was low, implying that the Q was in the oxidized state. In the presence of the powerful inhibitor of oxygenic photosynthesis, DCMU, only one quantum per 150 chlorophyll a_2 molecules sufficed for an appreciable fluorescence increase, or Q reduction. Since the concentration of Q was of the same order as that expected for reaction centers, and its redox state strongly affected the fluorescence yield of system 2, Q was thought to be part of the energy trap or reaction center of system 2 and to accept the hydrogen or, as now has been established, the electron from the hypothetical trap, P; about one DCMU molecule per Q prevented the oxidation of reduced Q by system 1, but not its reduction by system 2.

The characterization of the trapping pigment of system 2, P680, a chlorophyll *a* dimer, which because of space limitations cannot be discussed here, was accomplished later and involved the contributions of several research groups over a number of years. The acceptor side of reaction center 2 has been found to be homologous to that of purple bacteria, pheophytin and quinone corresponding to their bacterial counterparts. Also amino-acid sequence homologies have been observed.

Concluding remarks

The above description may be compared to a rough sketch for a highly detailed painting which seems to be not very far from completion. More than 90% of essential confirming, detailing and extending research, in our laboratory, let alone in other laboratories, has hardly been touched upon. Reviews on relatively recent research may be found in Refs. 25 and 26 and in the volume referred to in Ref. 15.

A few remarks on the function of reaction centers are in order. The reaction center is a protein spanning the photosynthetic membrane. P is imbedded in this protein at the outside of this membrane. Upon excitation of P an electron is transferred from P via intermediate electron acceptors to a final acceptor adjacent to the inner membrane space. In *R. rubrum*, for example, this acceptor is a quinone, Q, and the electron transfer in the reaction center results in the formation of the radicals P^+ and Q^-, which back-react in > 0.1 ms, a time sufficiently long to permit removal of the charge from P^+ by reduced cytochrome. In all photosynthetic organisms a similar separation and 'stabilization' of charges must precede the subsequent 'slow' dark reactions of photosynthesis, namely photophosphorylation and the hydrogen and electron transfer from the hydrogen donor (such as water in oxygenic organisms) to a reaction center and from this center (in oxygenic organisms via a second reaction center) to carbon dioxide.

As already mentioned, in addition to the two bacteriochlorophyll molecules of the dimer P, the reaction center of purple bacteria contains two bacteriochlorophyll molecules absorbing around 800 nm, B_1 and B_2, and two pheophytins, which are all possible electron transfer components. Some time ago we discussed a hypothetical membrane crossing electron transfer chain, embedded in the reaction center; this chain was assumed to consist of the two bacteriochlorophylls of P, one pheophytin and two quinones [21]. The 0.1 ms stabilization of $P^+ \ldots Q^-$ required theoretically a minimum loss of about 25% of the 'free energy' of excitation of P* in the reaction $P^* \ldots Q \rightarrow P^+ \ldots Q^-$; the real loss was estimated to be about twice as high. A rapid electron transfer through a chain of such molecules containing conjugated systems requires that the wave functions partly overlap, which implies that the shortest distance between two adjacent systems does not exceed a few Å. In order to avoid excessive energy losses in the form of fluorescence and internal conversion in the antenna molecules, in all photosynthetic organisms the excitation energy must be removed from P (and thus the primary reaction must occur) within 10 ps. Several research groups had earlier established by means of picosecond absorption difference spectroscopy and other methods that in reaction centers of purple bacteria an electron was transferred to pheophytin within a few picoseconds after excitation of P.

It is interesting to compare our early speculative model [21] with the picture of the reaction center shown in Fig. 3, Ref. 15, p. 378, based on the electron density distribution obtained from the X-ray data on *Rps. viridis*. This picture shows that one of the Bs is located between P and pheophytin; we had assumed that both Bs were located at the side of the electron transfer chain and possibly facilitated transfer of excitation energy from the antenna to P. Before the X-ray data had been obtained, Shuvalow [22] proposed on basis of a variety of data an arrangement of conjugated systems remarkably similar to that obtained from the electron density distribution; B_1 is located between P and bacteriopheophytin and may function as an electron transfer intermediate. Later he concluded from a small picosecond bleaching around 800 nm [23], that the electron passed indeed through B_1, but this conclusion is uncertain, since other investigators, who used a faster but 'noisier' method, were not able to observe this bleaching [24]. Already in 1952 the absorption difference spectrum due to B caused difficulties in interpretation, and

now it did so again on a picosecond timescale.

In other organisms, the electron transfer chains of reaction centers appear to be analogous to those in purple bacteria: the chains start with chlorophyll(s) in some reaction centers followed by pheophytin and quinone and in others by proteins containing iron-sulfur centers; the redox potentials of the primary donor couples P^+/P and of the final acceptor couples are different for various reaction centers, as are the types of chlorophylls, pheophytins and quinones for various groups of organisms.

Further experimental and theoretical studies, especially of the pico- and subpicosecond kinetics of the absorption spectra of reaction centers, may not only deepen our insight in the early electron transfer processes of photosynthesis, but also lead to important developments in quantum chemistry. By means of genetic engineering it may be possible to produce reaction centers modified for facilitating the study of the electron transfer process, or for constructing highly efficient solar cells or extremely rapid switching components in electronic chips. In my opinion, however, public financial support should be directed mainly to fundamental research until the relevant mechanisms are thoroughly understood.

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Reversible changes in bacteriochlorophyll in purple bacteria upon illumination

Illumination was found to bring about a reversible change in the absorption spectrum of a suspension of purple bacteria. The change was measured by means of a sensitive differential spectrophotometer. We constructed this spectrophotometer—previously described[1]—for the measurement of small changes in the absorption spectrum of light-scattering suspensions upon illumination.

The absorption vessel was a glass cylinder of 5 cm length and 2 cm diameter, sealed at

both ends with glass plates. An opening over the length of the cylindrical wall was covered with a glass plate, after the vessel was filled to the rim. Since this cover was not completely air-tight, the suspensions, which were not able to respire vigorously, were possibly not anaerobic. A weak monochromatic beam of variable wavelength for measuring the changes in absorption traversed the length of the vessel. The actinic beam was incident on the side wall of the vessel. Illumination of the suspension with a broad band in the blue caused a decrease in absorption at 880 mμ, which was completely reversed in darkness. Fig. 1 shows that, for a suspension of *Rhodospirillum rubrum* in water, the absorption decreased roughly linearly with light intensity up to a certain saturation value, while, for bacteria suspended in a solution of 0.5 % peptone and 0.5 % NaCl, a change in absorption occurred only at intensities at which photosynthesis presumably was saturated.

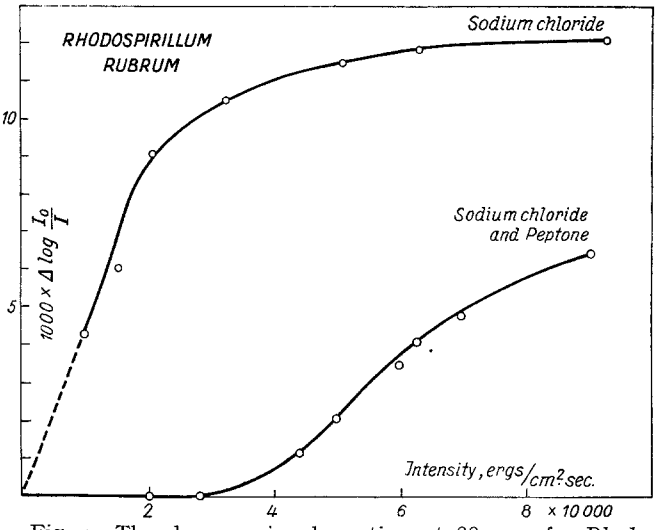

Fig. 1. The decrease in absorption at 880 mμ for *Rhodospirillum rubrum* strain 4 is plotted as a function of the intensity of the exciting light, which occurred in a band between 400 and 550 mμ. The bacteria were centrifuged and suspended in tap water containing 0.5 % NaCl and 0.5 % peptone plus 0.5 % NaCl respectively.

The changes were measured at various wavelengths at a constant intensity of actinic light, somewhat lower than needed to attain saturation. These changes, when plotted as a function of the wavelength, determine a difference spectrum (Fig. 2). The long wavelength peak of this spectrum occurs at approximately the same wavelength as the maximum of the type of bacteriochlorophyll called B 890[1] and thus probably is caused by a decrease of B 890 absorption upon irradiation. This decrease can be considered to be caused either by complete disappearence at the maximum of a small fraction of the bacteriochlorophyll or by a small change in the absorption spectrum of the total amount. If the absorption spectrum of a small fraction disappears completely, then the decrease in absorption of *Rhodospirillum* at 810 mμ is too great to be caused exclusively by disappearance of B 890. Since previous evidence[1] suggested that the infrared absorption of *Rhodospirillum* was caused by B 890 only, the changes were thought to be caused by a small change in an appreciable fraction of B 890. Very recently, however, THOMAS, GOEDHEER AND KOMEN[2] found unexpectedly that treatment with acid decreased the absorption at 800 mμ somewhat more than that of the major maximum. This suggested that part of the absorption at 800 mμ was caused by a trace of another pigment, perhaps B 800. Thus part of the decrease at 810 mμ might be caused by a disappearance of the minor absorption maximum of a small fraction of B 890 and of the major maximum of a fraction of B 800. The observation of a pronounced decrease at 810 mμ in the absorption spectrum of *Chromatium* (Fig. 2), a species which contains a larger amount of B 800 than *Rhodospirillum*, supports the suggestion that the peak at 810 mμ is partly caused by B 800. The difference spectrum in the visible of *Rhodospirillum rubrum* suspended in aerobic water showed that illumination produced an absorption band at about 430 mμ[4]. Also in anaerobic peptone, but at actinic intensities far above that needed for saturation of photosynthesis, an increase in absorption was found at 430 mμ which was superimposed upon a decrease in absorption which occurred already at lower intensities and indicated oxidation of a cytochrome.

Since the increases in absorption at 790 and 430 mμ upon illumination are most pronounced in oxidizing media[4,5], these increases are presumably caused by an oxidation. This oxidation might be the removal of the two hydrogen atoms from the fourth pyrrole nucleus of bacteriochlorophyll. Treatment of a methanol solution of bacteriochlorophyll with ferric chloride oxidized bacteriochlorophyll to a chlorophyllous pigment—possibly bacterioviridin—with major absorption peaks in ether at 434 and 676 mμ (*cf.*[6]). The blue maximum corresponds fairly well with the maximum of the difference spectrum, but the red maximum differs about 120 mμ from the infrared peak in illuminated bacteria. Such a shift might well be caused by the solvent: a shift of 120 mμ was observed in the infrared maximum of B 890 in *Chromatium* upon extraction with ether[3], while the near ultraviolet maximum is less affected. The absorption maximum of bacterioviridin in green bacteria is about 747 mμ, corresponding to a shift of 70 mμ (*cf.*[7]).

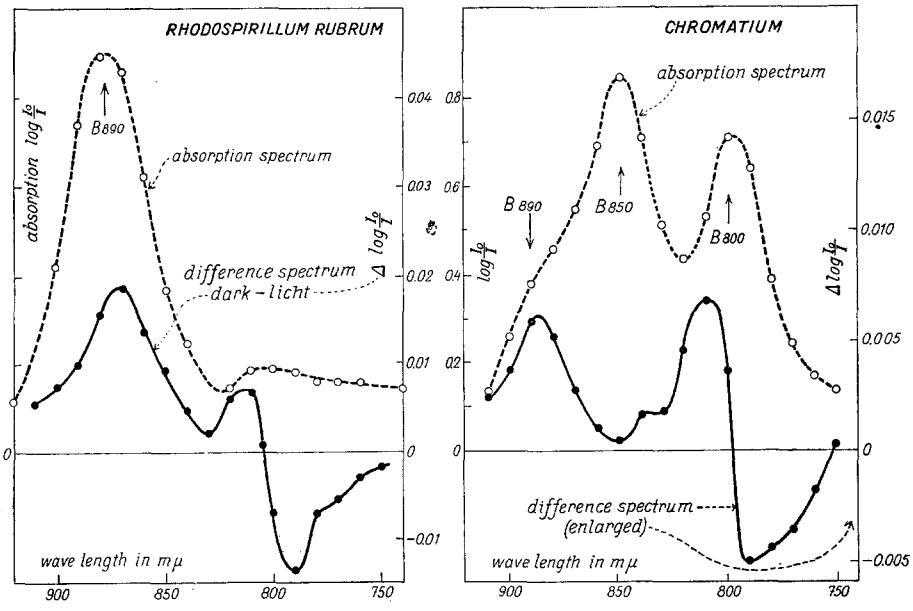

Fig. 2. Absorption and difference spectra for *Rhodospirillum rubrum* strain 4 suspended in tap water, and for an aqueous extract[3] of *Chromatium* strain D. The difference spectra are enlarged 10 and 50 times with respect to the absorption spectra. The spectral changes were brought about by illumination with an intensity of $3 \cdot 10^4$ ergs/cm^2.sec in the region 400–550 mμ. The location of the main infrared maxima of the various bacteriochlorophyll types is indicated by arrows.

The change in bacteriochlorophyll in illuminated bacteria was so far observed only when the cytochrome pigment was already in the oxidized state[4]. The reduced cytochrome may react so fast with oxidized bacteriochlorophyll that the concentration of oxidized bacteriochlorophyll is too small to be observable.

These observations suggest the following reactions:

Excited bacteriochlorophyll + X $\longrightarrow$ oxidized bacteriochlorophyll (bacterioviridine?) + XH (photosynthetic reductant).

Oxidized bacteriochlorophyll + reduced cytochrome + proton(s) $\longrightarrow$ bacteriochlorophyll + oxidized cytochrome.

It should, however, be pointed out, that this is not the only possible interpretation.

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