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A COOPERATION OF TWO PIGMENT SYSTEMS AND RESPIRATION IN PHOTOSYNTHETIC LUMINESCENCE

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SUMMARY

Luminescence kinetics of photosynthesizing cells were investigated. This was done by measuring afterglow as a function of intensity and wavelength of actinic light as well as of temperature. In order to explain the chromatic transients, induction effects, and various other aspects of luminescence, the presence of a chloroplast respiratory "r" system was postulated. A "feedback" of products formed by photochemical "p" and "q" systems, described earlier, into the dark "r" system is believed to affect the state of reduction of cytochrome and, with it, luminescence.

The relation of luminescence to gas-exchange measurements and a possible explanation of various aspects of photosynthesis by interaction of the "p", "q" and "r" systems is discussed.

INTRODUCTION

In a preceding paper[1] it was concluded that chlorophyll afterglow, measured by the circulating-flow method, is an indicator of the state of reduction of certain intermediates in photosynthesis. Measurement of afterglow thus provides a tool for studying photosynthetic kinetics *in vivo* under conditions approaching those of continuous illumination under a wide range of light intensities.

Luminescence was found to be emitted by only one pigment system (the promoting or "p" system). It is believed to be emitted by chlorophyll *a*, excited as a result of the recombination of products from water photolysis. Light absorbed by a second pigment system (the "quenching" or "q" system) results in a decrease in luminescence evoked by the "p" system. This was explained in terms of a removal of electrons from the "p" system, thus preventing recombination. Light absorbed by the "p" system was assumed to oxidize water and reduce a chemical compound X (possibly plastoquinone) and a cytochrome (probably cytochrome *f*), whereas light absorbed by the "q" system was supposed to oxidize this cytochrome and reduce a pyridine nucleotide. Combined action of both systems is needed to produce full photosynthesis, the luminescence "quenching effect" thus being inversely related to the photosynthetic "enhancement" effect.

Abbreviations: PN, pyridine nucleotide; PNH, reduced pyridine nucleotide; (CHO), carbohydrate.

Changes in the state of reduction of cytochrome during illumination will affect the intensity and decay characteristics of the afterglow. With this in mind the following phenomena are studied: the luminescence transients after a change in light intensity, upon variation of the spectral composition of light (chromatic transients), upon the onset of illumination (induction effects) and on change in the sequence of illumination effects. In this way information about the kinetics of photosynthesis in undisturbed systems is obtained under a wide range of light intensities and temperatures.

METHODS

The experiments were performed using a circulating-flow method and under the conditions described in a previous publication[1]. Intensities of white light are given in ergs/cm²sec covering the spectral region between 400 and 700 mμ.

RESULTS

Luminescence transients due to a sudden change in intensity of white exciting light

A sudden change in intensity of exciting light, due to addition or removal of neutral (reflexion) filters, results in marked transients of luminescence intensity.

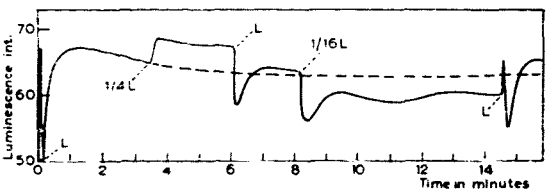

Fig. 1. Luminescence transients resulting from changes in intensity of white exciting light. L indicates maximum light intensity ($2 \cdot 10^4$ ergs/cm² sec), $^1/_4$ and $^1/_{16}$ L is produced by addition of neutral reflexion filters in the light beam.

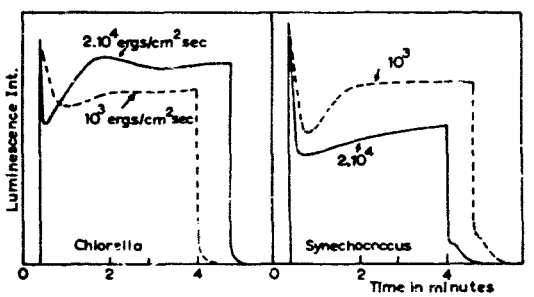

Fig. 2. Luminescence induction curves of Chlorella and Synechococcus at different intensities of white incident light and at 25°. Note the bend in the decay curves of Synechococcus luminescence, indicating two different decay times. Removal of some far red light by interposing a weak copper sulfate filter results in a change of the shape of Synechococcus induction and decay curves approaching those of Chlorella.

In Fig. 1 such transients are presented for *Chlorella pyrenoidosa*. In the depicted experiment, luminescence intensity is optimal at an actinic light intensity of the order of $4 \cdot 10^3$ ergs/cm²sec. The equilibrium value of luminescence in some cases is reached by a series of damped oscillations. Similar transients were observed with the blue-green alga Synechococcus. In this species the increase in luminescence following a decrease in intensity of incident light is much more marked.

Luminescence induction phenomena in white light

Luminescence shows marked induction phenomena at the start of illumination. The shape of induction curves may show considerable variations with general con-

ditions of illumination, temperature, CO_2 content, growth conditions and light and dark pretreatment. Fig. 2 gives induction curves of Chlorella and Synechococcus in white light from incandescent lamps at different intensities and at 25°. At low temperatures (5–10°) usually only the first luminescence peak is present.

Luminescence induction obtained by light absorbed in "p" and "q" systems simultaneously

A decrease in luminescence, resulting from addition of light absorbed in the "q" system to light absorbed in the "p" system, is often preceded by a sharp positive peak of luminescence. In Fig. 3 such a peak is demonstrated with Chlorella. The presence of this peak is assumed to be due to the fact that at the "quenching wavelength"

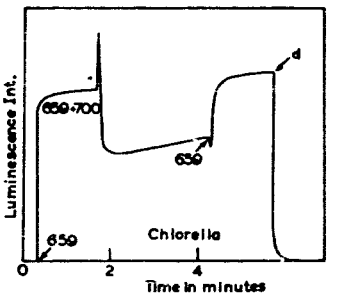

Fig. 3. Luminescence chromatic transient with Chlorella at intensities of exciting light of about 200 ergs/cm²sec. The sharp increase in luminescence occurring if 700-mμ light is added to 659-mμ light is almost absent if 710-mμ light is used instead of 700-mμ light.

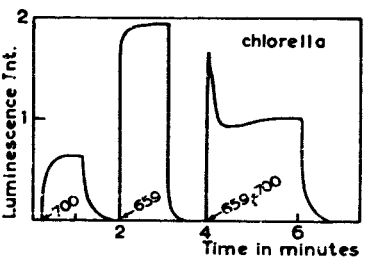

Fig. 4. Luminescence of Chlorella resulting from illumination of 700 mμ and 659 mμ applied successively and simultaneously. If 659 mμ is applied first, instead of 700 mμ, no induction occurs when both beams are applied simultaneously. If a dark period of 20 min occurs between application of 659 mμ and the combination of both beams, only a very weak induction peak is measured.

used (700 mμ), part of the light is absorbed by the "p" system (absorption in the long wavelength "tail" of "p" chlorophyll *a*). Absorption by this fraction results in an increase in luminescence. The decrease in luminescence brought about by absorption in the "q" system at this wavelength occurs rather slowly at the light intensities used in the experiment shown. Hence a temporary increase in luminescence, the positive peak, results. The observation that no positive peak occurs if the added "*q*" light is of sufficiently long wavelength (and equal amount of absorption) to make the fraction of "p" absorption negligible, is in agreement with this hypothesis.

With Synechococcus, where the "q" system includes also the major fraction of chlorophyll *a* with maximum absorption at 680 mμ, a small positive peak usually is observable only upon addition of light between 670 and 680 mμ.

If this brief increase in luminescence preceding the luminescence "quenching effect" is to be explained by a difference in kinetics of "p" and "*q*" systems, this explanation may also be valid for luminescence induction phenomena. In Fig. 4 the luminescence time curve is presented for successive and simultaneous excitation of the "q" (700 mμ) and the "p" (659 mμ) systems. A high peak prevails if the combination of both beams is given shortly after the "promoting" light. No peak, however, occurs if the "q" light is given shortly before the combination of both beams. If the combination of both beams is given after a prolonged dark period (20 min), a weak in-

duction peak is visible, irrespective of the order of application of "p" and "q" light before this period. The intensity of both beams was of the order of 150–200 ergs/cm²sec.

These observations can also be explained by the assumption that the luminescence phenomena reflect reduction and oxidation of cytochrome (*cf.* ref. 1). If a large number of cytochrome molecules are reduced by "p" illumination, a second illumination by combined "p" and "q" light will result in a decrease in the number of reduced cytochrome molecules. As reduced cytochrome is assumed to be connected with high luminescence values, a decrease in luminescence, the induction peak, can be expected to occur. If, on the other hand, all cytochrome is in the oxidised state due to "q" illumination, a second illumination by combined "p" and "q" light will result in an increase in the number of reduced cytochrome molecules. Under these conditions an increase in luminescence with time, rather than an induction peak, is expected. Such an increase is actually observed.

The occurrence of a very weak induction peak with Chlorella after 20 min dark storage, irrespective of the order of application of "p" and "q" light, indicates that dark oxidation–reduction of cytochrome occurs which establishes an equilibrium after this period.

With the blue-green alga Synechococcus usually little or no effect of "p" or "q" pre-illumination was found. This may indicate that the dark oxidation system is much more active than with Chlorella.

Although with combination of light absorbed by the "p" and "q" systems in Chlorella a peak of luminescence induction may be produced, the induction curves in white light of high intensities are more complicated. The following observations are relevant to this aspect.

Dependence of the "critical intensity for quenching" upon pretreatment and temperature

A decrease in luminescence due to addition of "q" light to "p" light does not occur at low intensities of actinic light. If the intensities of both "p" and "q" beams are reduced to the same percentage, the "quenching effect" disappears below a certain light intensity. This "critical intensity for quenching" was found to depend upon various parameters, such as pretreatment of algae, age of suspension, conditions of growth, and species. A prolonged dark incubation or a decrease in temperature results in a decrease in "critical intensity for quenching". With Chlorella at room temperature (25°) it usually is found to be low (about 20 ergs/cm²sec), while Synechococcus showed a much higher value (100–200 ergs/cm²sec). In some experiments the "critical intensity for quenching" was so high that with the light intensities obtainable with interference filters in our experiments (maximal about 300 ergs/cm²sec) no luminescence "quenching effect" could be detected. In all experiments with photosynthesizing cells, however, which were made with the green algae Chlorella and Euglena, the blue-green algae Synechococcus and Anacystus and the red alga Porphyridium, this effect could be detected if the promoting light consisted of white light from which long wavelength red light had been removed by interposing a copper-sulfate filter, while the "quenching" light beam consisted of only long wavelength red light (obtained by addition of a Schott Rg8 filter to the white light beam with the green algae or an RG5 with blue-green or red algae).

The dependence of "critical intensity for quenching" on these parameters and the results of the preceding section lead us to suggest that in photosynthesizing cells a

system is present which results in a dark oxidation of cytochrome. A decrease in luminescence due to "q" absorption will then be observable only if cytochrome is reduced by "p" absorption at such a rate that chloroplast dark oxidation cannot cope with all of it. With Chlorella, under the usually encountered conditions, this dark oxidation system should be weak, while with the blue-green algae Synechococcus and Anacystus its value should be ten times higher.

A phenomenon, observed during polarographic oxygen determinations, should be mentioned here. It was found that prolonged dark incubation resulted in a marked decrease in respiration of Synechococcus and, to a smaller degree, also of Chlorella. Respiration was stimulated by subsequent illumination. Other investigators also found an increase in respiration upon illumination after dark incubation[2]. This leads us to assume that the dark oxidation system represents some kind of chloroplast respiration ("r") system. As "p", "q" and "r" systems all are assumed to act upon cytochrome, the chloroplast "respiration" also will be influenced by light absorption. Then a "feed-back" of electrons, from products arising from the photochemical "q" system, into the dark "r" system will affect the state of reduction of cytochrome and hence will be observable in luminescence.

Luminescence chromatic transients in Synechococcus

As shown in Fig. 5, the chromatic transients occurring with Synechococcus as a result of addition of "q" light (680 mμ) to "p" light (615 mμ) differ from those of Chlorella. The initial spike is only weak, but luminescence, after decreasing, resumes its previous value after a minute's illumination. This effect, which is much more marked at 25° than at 15°, may be seen as resulting from the mentioned "feed-back" mechanism.

Induction phenomena resulting from interaction of "p", "q" and "r" systems

Addition of "q" light to "p" light at relatively low light intensities results in a luminescence induction peak in Chlorella. The luminescence induction phenomena

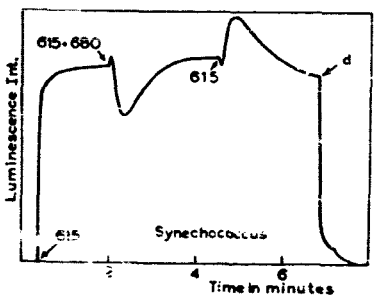

Fig. 5. Chromatic transients with Synechococcus. Luminescence decreases due to addition of 680-mμ light, but increases to approximately the previous values. A similar, but negative, effect occurs after removal of additional light. Light intensities are of the order of 200 ergs/cm²sec. The sharp peak of luminescence increase is much weaker than with Chlorella.

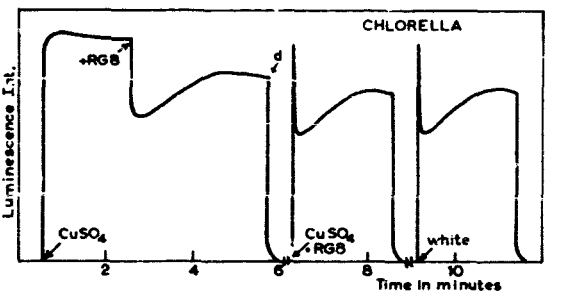

Fig. 6. Induction effects with Chlorella obtained by addition of long wavelength red (685 mμ) to white light from which long wavelength red has been removed (1 cm 10% $CuSO_4$ filter). Neither beam applied on its own results in appearance of induction phenomena; simultaneous application of both beams results in the same luminescence induction as occurs in one beam of white light. At these intensities an increase in luminescence after a decrease due to addition of "quenching light" is observed also with Chlorella.

in white light at higher intensities, however, are more complicated. At these intensities a marked second wave appears in the luminescence induction curve, sometimes followed by one or more waves of decreasing amplitude. Fig. 6 indicates that very little induction occurs if white light is given without far red light (using a $CuSO_4$ filter). A strong "quenching effect" occurs upon subsequent addition of long wavelength red light ("q" absorption: isolated from white light by an RG8 filter). Luminescence, however, recovers partly after the decrease as occurs with [illegible] at lower light intensities (*cf.* Fig. 5). Neither beam given apart results in induction phenomena, but simultaneous irradiation (Fig. 6) results in induction phenomena nearly identical with those obtained with one beam of white light. The second wave of luminescence induction thus seems coupled to addition of high intensity "q" light, and may well be interpreted as being due to the "feed-back" between "q" and "r" systems, mentioned previously

Induction and luminescence "quenching" of chloroplast suspensions

Luminescence of isolated chloroplasts (like chloroplast fluorescence) as a rule does not show marked induction phenomena at high intensities of incident light. A luminescence "quenching effect" due to addition of long wavelength absorbed light could be observed only at temperatures below 17° if monochromatic light isolated by interference filters was used. The mentioned effect, even at low temperatures, could not be observed for more than 10–20 min. At room temperature the effect could be observed for a short period only if high intensities of mainly "q" light (RG8 filter) were added to mainly "p" light ($CuSO_4$ filter). Weak induction effects could only be seen during the period in which the luminescence "quenching effect" could be observed.

Luminescence induction with photosynthetic bacteria

Neither a marked luminescence induction nor a luminescence "quenching effect" was observed with luminescence of *Rhodospirillum rubrum*. As mentioned in an earlier publication[1], luminescence at room temperatures of these bacteria is very weak. It was believed to be emitted by a pigment system assumed to be equivalent to the "q" system of green plants. In agreement with this hypothesis, it was found that addition of oxidants (ferricyanide, quinones) resulted in an increase in luminescence. This phenomenon is in contrast to the occurrence of luminescence in green plants, where the afterglow is emitted by the "p" system and addition of oxidants results in a decrease in luminescence.

DISCUSSION

The assumption of the presence of some kind of chloroplast respiratory system appears to fit in with a number of experimental results apart from luminescence.

Stimulation of respiration, as a result of illumination, has been measured mainly on the oxygen side by a polarographic method[3,4]. Results of BROWN AND WEBSTER[5] obtained with the mass spectrometer show that with the blue-green alga Anabaena stimulation of respiration during illumination can be measured, provided high intensities of white light are used. BROWN[3] did not detect an influence of illumination on oxygen consumption with Chlorella at atmospheric oxygen pressure. This seems to be consistent with our findings, that as a rule the "critical intensity for quenching"

was much lower and the time needed for dark oxidation much longer with Chlorella than with the blue-green algae Synechococcus and Anacystus.

BROWN AND WEISS[6] found that, with the green alga Ankistrodesmus, photostimulation of respiratory oxygen uptake occurred only in strong light, while light induced an inhibition of CO_2 evolution which was nearly independent of light intensity.

JAMES AND DAS[7] concluded from their measurements with preparations of purified bean chloroplasts that no chloroplast respiration occurred in their preparations. Their figures, however, indicate a small but definite oxygen uptake in the dark even with highly purified chloroplasts. The observation that this residual respiration is not enhanced by addition of hydroquinones (contrary to mitochondrial respiration), seems not to contradict our assumption. The relatively weak chloroplast respiration might be affected differently from mitochondrial respiration.

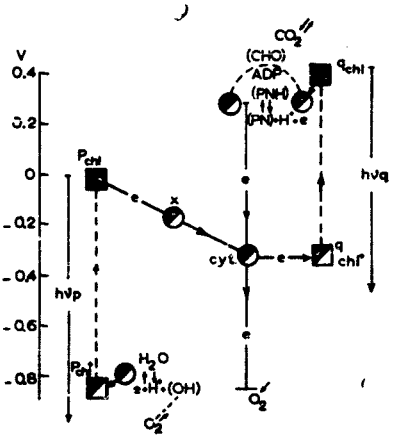

Fig. 7. Hypothetical energy scheme of photosynthesis in which two photochemical systems ("p" and "q") are cooperating with a dark respiration "r") system. Electrons, brought to the carbohydrate potential level as a result of "q" absorption, are assumed to participate in the "r" system. With the "feed-back" system thus obtained, changes in the cytochrome reduction level are produced, which may account for transients and induction phenomena. The biochemical sign convention has been followed. To visualize the picture as an electron flow, the sign has to be reversed.

In order to illustrate the fluctuations in the state of reduction of cytochrome, which are assumed to be responsible for the fluctuations in luminescence, a chloroplast respiratory system has been incorporated in the hypothetical energy scheme given in Fig. 7. Chloroplast respiration in this scheme is depicted as the electron flow driven by a battery of about 1.5 V whose anode and cathode are represented by O_2 and PNH, respectively. If both a respiratory PNH and an O_2 molecule are present, short circuiting in the respiratory "r" chain occurs and water is formed from O_2, H^+ and electrons at the oxygen electrode. At the other electrode PN and H^+ are formed.

Thus the following events may occur: (a) an oxygen molecule diffuses to the "O_2" electrode, resulting in the removal of an electron from the schematic "r" wire, *i.e.* cytochrome is transformed into its oxidised state. (b) A new PNH molecule is produced (chemically, from some (CHO) compound, with release of CO_2). From this PNH an electron is fed into the schematic "r" wire, *i.e.*, cytochrome is transformed into its reduced state.

The frequency with which a cytochrome molecule is reduced or oxidised depends upon the rate of "arrival" or "removal" of the end products. The average potential of all cytochrome molecules in the chloroplast in the dark will be determined by the influx ratios at both ends of the circuit.

At atmospheric oxygen conditions this average cytochrome potential most

probably will be on the oxidised side of the redox potential; in anaerobic conditions it will be on the reduced side.

The latter conditions will result in the following phenomena. At low light intensities absorption in the "p" system will be ineffective. Reduced X can no longer reduce cytochrome and energy is re-emitted as luminescence. Absorption in the "q" system effects, it is true, oxidation of cytochrome, but this cytochrome will be reduced by respiratory PNH before it can be reduced by the excited [illegible]. Thus, in anaerobic conditions, no oxygen evolution will occur at low light intensities.

At higher light intensities, so much cytochrome is oxidised by "q" absorption, that the electron of the excited "p" system has a fair chance to find an oxidised cytochrome with which to react. In fact, the probability of "simultaneous" excitation of "p" and "q" systems acting on the same cytochrome increases as the square of the light intensity. Oxygen production in the "p" system occurs and anaerobic conditions are soon replaced by aerobic ones, resulting in normal photosynthesis. Oxygen in our scheme thus is not required in the photosynthetic process, as it was assumed by WARBURG[8]. In this respect it should be mentioned that ALLEN[9] observed photosynthesis at O_2 pressures even below 10^{-6} mm Hg. If the cells are placed in a stream of oxygen-free nitrogen, the oxygen pressure in the cell will never be high, even at full illumination. As a consequence the average cytochrome potential will stay on the reduced side and will not reach its optimum value for full photosynthesis. In such a case only a weak maximal photosynthesis may occur, as many of the electrons liberated from water by "p" absorption do not find an oxidised cytochrome to react with. In this way the results of FRANCK, PRINGSHEIM AND LAD[10] showing a low residual photosynthesis in a stream of oxygen-free nitrogen, with saturation reached by a second power curve, might be explained without the assumption of a metabolic poison produced by anaerobiosis.

An additional result of anaerobiosis might be the production of molecular hydrogen due to illumination. At low light intensities the electron produced by "q" chlorophyll as a result of excitation cannot be used in formation of PNH, as all PN present in the cell is expected to be in the reduced state in the dark under these conditions. Then hydrogen evolution might occur, following $2e + 2H^+ \rightarrow H_2$ (see refs. 11, 12).

According to Fig. 7, the quantum requirement of photosynthesis is expected to be at least 8, four quanta being needed in each photochemical step. This minimum value was found by EMERSON AND LEWIS[13] and others in the great majority of quantum requirement experiments. At very low light intensities, however, absorption by the "q" system is inactive as cytochrome reduced by "p" absorption is predominantly oxidised by the "r" system, the probability of "simultaneous" excitation of "p" and "q" systems being low at these intensities. It does not seem impossible to increase chloroplast respiration by special pretreatment and experimental conditions to such a degree, that the "p" system only is responsible for oxygen production in an intensity region far beyond 20 ergs/cm²sec (the "critical intensity for quenching" with Chlorella, or higher values with blue-green or red algae). Under these conditions, and provided light absorbed at wavelengths where absorption in the "p" system predominates, a quantum requirement of 4, as measured in WARBURG AND NEGELEIN'S[14] original experiments, is to be expected. These low values could be obtained by these authors only by adherence to highly specialised growth conditions and very weak illumination.

In this context it should be mentioned that KOK[15] found a rather sharp break in the photosynthesis light curves of Chlorella around the point of respiration compensation, the quantum requirement values below and above this point being 4 and 7 approximately.

Luminescence and fluorescence behave similarly in many respects. It was found that luminescence and fluorescence induction curves were similar in shape, provided the same light intensities, spectral composition of the light, CO_2 concentration and pretreatment of the cells were used. Also, we found that heating of a chloroplast suspension to 65°, which almost completely abolishes luminescence, decreases fluorescence to less than 25 % of its previous value (*cf.* ref. 16). Intensity transients, as shown in Fig. 1 with luminescence, are known from the literature to occur also with fluorescence[17]. Spectral investigations of fluorescence induction phenomena of bean and Aspidistra leaves demonstrated that induction phenomena were much more marked with fluorescence measured at 680 mμ than with fluorescence measured at 720 mμ. This suggests strongly that fluorescence induction phenomena also are correlated with emission from the "p" system.

A coupling of "q" and "r" systems is indicated further by the following results. The increase in luminescence following a decrease due to addition of "q" light to "p" light (as shown in the luminescence transients of Synechococcus and at much higher light intensities also of Chlorella) may be assumed to be brought about by an increase in electron flow through the "r" system resulting from a common (CHO) pool of "r" and "q" systems. Due to this increase more cytochrome is reduced. As a consequence the number of back reactions in the "p" system, resulting in luminescence, increases. A greater rate of back reactions may be expected to coincide with a decrease in photosynthetic oxygen production. BLINKS[18] and MYERS AND FRENCH[19] who applied the light beams alternately instead of simultaneously, observed chromatic transients in oxygen production. FRENCH AND FORK[4] measured the action spectrum of the increase in oxygen consumption after illumination. This action spectrum appears similar to that of our "q" system These results may be explained by a temporary increase in electron flow through the "r" system due to a high concentration of common (CHO) produced by "q" absorption. Such an increased electron flow will result in an increased oxygen uptake.

Light absorbed by "q" chlorophyll *a* in our scheme is unable to bring about the splitting of water. At all wavelengths, except in the extreme red part of the spectrum, "p" and "q" absorption are overlapping. Hence, in the extreme red photosynthetic activity is low (long wavelength decline), while this activity can be enhanced by the addition of some "p" light ("EMERSON" or enhancement effect). This enhancement effect does not occur below a critical light intensity[20]. As mentioned before, the photosynthetic enhancement effect may be seen as inversely related to the luminescence "quenching effect".

Due to an increase in chloroplast respiration resulting from absorption in the "q" system, maximum oxygen production at saturating light intensities in the far red region of the spectrum is expected to decrease, instead of being independent of wavelength as should occur if only the "p" and "q" system were present. Such a decrease indeed was measured by MCLEOD[21]. It remains to be seen whether the 730-mμ inhibition effect, too,—measured as a decrease in photosynthesis if extreme red is added to red light—observed by GOVINDJEE, THOMAS AND RABINOWITCH[22]

might not be explained in terms of stimulation of respiration by "q" light.

Another observation, which is in agreement with our hypothesis is the following. In blue-green and red algae, where "q" absorption amounts to an appreciable percentage of total absorption and chloroplast respiration seems to be relatively high, no horizontal photosynthetic saturation level is reached, but oxygen production as a function of light intensity drops markedly after having reached a maximum. This occurs without noticeable damage to the photosynthetic apparatus[23].

Also, various phenomena known from measurements of photosynthetic and fluorescence induction to occur seem explicable by the hypothesis of a cooperation of "p", "q" and "r" systems. VAN DER VEEN[24] showed that under certain conditions induction curves of CO_2 uptake show damped oscillations, such as may occur in a "feed-back" mechanism. Similar oscillations were measured by us with oxygen uptake of bean leaves. They may also be measured with luminescence or fluorescence, provided sufficiently high illumination intensities are used. According to MCALLISTER AND MYERS[25] curves of CO_2 uptake and fluorescence at these intensities are almost mirror symmetrical. Usually the conditions of illumination of photosynthetic cells are such, that the first peak of the damped oscillations in the gas exchange curves (corresponding to the second maximum in the luminescence and fluorescence curves) is highly dominant (aperiodic conditions). An initial carbon dioxide uptake (or oxygen burst) is then observed. By heating or cooling the cell suspension such a carbon dioxide uptake may be separated from continuous photosynthesis[24]. This might be caused by damage to the mechanism by which the primary carbohydrate (CHO) is removed. Photosynthesis in that case stops as soon as all photosynthetic PNH, reduced by the "q" fraction of actinic light, has produced (CHO), resulting in an initial CO_2 uptake. In the dark, after the end of illumination, chloroplast respiration results in an oxidation of (CHO), thus producing a CO_2 "gush" In agreement with this theory is the observation of VAN DER VEEN that at 0°, when chloroplast respiration is assumed to be very weak, the gush in the dark is absent.

With Chlorella, EMERSON AND LEWIS[13], BROWN AND WITTINGHAM[26] and others observed a gush of CO_2 at the start of illumination, instead of a CO_2 uptake as occurs with most other species. Little or no oxygen effects coincided with this burst. In this case the explanation might be seen in a stimulation of chloroplast respiration due to cytochrome oxidation effected by the "q" fraction of white light, here preceding photosynthetic CO_2 uptake. If the low rate of chloroplast respiration of Chlorella is assumed to be caused by limited diffusion on the O_2 side, oxygen uptake might be little affected.

With the photosynthetic bacterium *Rhodospirillum rubrum* no marked induction phenomena were observed. It is therefore suggested that mainly a single pigment system, the "equivalent q system", is active in luminescence. The possibility that an "r" system occurs in some photosynthetic bacteria should not be excluded. JOHNSTON AND BROWN[27] found a 60–80% inhibition of respiratory oxygen uptake in *R. rubrum* as a result of illumination.

Although the energy scheme for green plant photosynthesis, as presented above, is no doubt highly oversimplified, many of the kinetic properties of photosynthesis appear to be explicable in terms of a cooperation of the suggested "p", "q" and "r" systems.

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