either by chlorophyll itself, or by one of the accessory pigments. The interpretation of this highly unexpected result could be sought either in different photochemical functions of the several cell pigments, which must be combined to achieve photosynthesis, or in the existence of 2 or more different forms of excited chlorophyll a—one of which results from direct absorption in the far-red part of the spectrum, while the other can be obtained either by direct absorption of higher frequency quantas by chlorophyll, or by resonance energy transfer of these quanta from the excited accessory pigments. The first interpretation left puzzling the observed parallelism between the action spectra of photosynthesis and of chlorophyll fluorescence; the second left unexplained the striking difference in the position of the red drop in green and in red algae. Thus, the problem remains open, and calls for more experimentation, with the skill and patience Emerson would have applied to it.

In the midst of these experiments, Bob Emerson met sudden death when the plane, carrying him to a conference at Harvard University, missed the LaGuardia runway and plunged into the East River. As part of his dislike of new gadgets, Emerson distrusted airplanes and always advised me against flying. Only in the last few years, when his favorite train from Indianapolis to New York was discontinued, did he grudgingly choose air transportation for his trips to New York. He was booked for another flight, but the lateness of the ill-fated Electra in leaving Chicago made it possible for him to transfer to it at the last moment, hurrying him to his death.

To me, the death of Robert Emerson means the loss of a warm, steady and reliable friend, whose scientific advice was invaluable for me and my students, and for whose opinions in all fields of human interest I had the greatest respect, even if I did not share some of them. The feelings of his numerous and widely-scattered friends are well expressed in a letter from his Harvard friend, Kenneth Thimann, who wrote: "Bob is not a man whom you can ever forget. In some way Bob was the very symbol of uprightness; he loved the truth just as much as he loved the underdog, and he scorned the untruthful and could not have anything to do either with it or with the man who promulgated it. I can imagine his students feeling that they have to judge their lives by what Bob would have done in the circumstances . . . . Everyone who has come into contact with Bob must have been inspired by him to some degree; it is impossible not to be, just as it is impossible not to remember with clarity his every gesture, his ready smile—often belying fierce disagreement—his enormous ability for friendship and real tenderness. This is a kind of immortality—at least survival for another lifetime—in the memories and even to some extent in the characters of other people, which it is given to very few men to achieve."—EUGENE RABINOWITCH.

LIGHT INDUCED ABSORPTION CHANGES IN PHOTOSYNTHETIC ORGANISMS. II. A SPLIT-BEAM DIFFERENCE SPECTROPHOTOMETER[1]

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Previously (9 to 12), we described a method to observe relatively short lived absorption changes (i.e., having a minimum life time of some 5 milliseconds), induced by brief flashes of strong light.

The sample was intermittently cross-illuminated by a strong actinic beam and its momentary optical density was observed immediately before and immediately after each flash. With a few exceptions (e.g., the 520 shift in green cells and the infra-red effects in purple sulfur bacteria), the absorption changes occurring are so small that the effect of a single flash cannot be observed directly. This difficulty was overcome by the use of a rotating sector disc arrangement, which yields a steady sequence of flashes. A series of transmission values, before and after the individual flashes, then could be collected and averaged so as to yield significant data, even under severe conditions of signal to noise ratio. The rotating disc was, moreover, arranged in such a way that the photocathode was darkened each time a flash hit the sample. Neither scattered actinic light, nor fluorescence induced by it, interfered with the measurements, and the method, therefore, allowed full freedom to vary intensity and color of the actinic beam regardless of the wave length of the light detecting the absorption changes.

Another obvious advantage is the fact that the two observations to be compared are made with a single beam, so that small intensity fluctuations, settling of algae, etc. have relatively slight influence on the readings.

However, a serious limitation of this arrangement was that only variations of absorbance could be observed, which were either reversible (e.g., fast increase by each flash and slow decay in the dark periods) or additive (e.g., increase during each flash without restoration in dark).

[1] March 23, 1959.

Moreover, in most cases it was impossible to discriminate these two types of effects. We therefore felt the need for a better study of slowly occurring absorption changes, i.e., those on top of which eventually the fast phenomena might occur. Especially in the red spectral area—of most importance to photosynthesis—several incongruences exist among observations of "slow" absorption changes by other workers (1, 3, 9) and with our own earlier data on fast changes.

Adhering to the principle of time separation between actinic and detecting beams, we redesigned our apparatus so that a fraction of the detecting beam is guided around the sample and can be used as a reference. Reference and sample beam can be adjusted for equality before actinic illumination is applied. An inequality caused by absorbance changes induced by subsequently given actinic light can then be measured on an absolute rather than a relative basis.

Description of the Apparatus: A schematic illustration of the modified apparatus is shown in figure 1. The sample, contained in a small reaction vessel (R), is cross-illuminated by a flashing actinic beam (arrows) and a continuous detecting beam (dotted) leaving the grating monochromator (Mo). The monochromatic beam is split with the aid of 2 thick hemispherical lenses, ground and positioned as indicated: LL_1. The components of LL_1 are of the type used as a front lens in a microscope condenser (Bleeker Co., Zeist, Holland). Part of each is ground away in order to yield a flat side at an angle of 120° to the front plane. One of these lenses images the monochromator exit slit on the reaction vessel (beam AB), the other images the slit on a photographic circular density wedge W (beam W). The 2 beams are collected and made parallel by a 2nd pair of hemispherical lenses, LL_2, and pass 2 coaxial discs (D_s, D_f). About one third of each lens is ground away perpendicularly to the front to yield a flat side. A thin copperfoil separates the lenses of each pair (i.e., LL_1 and LL_2) and prevents the 2 beams from interfering with each other. A lens of larger diameter, placed closely behind the discs, finally reunites both beams on the cathode of the photomultiplier (P_m). The 2 beams can be adjusted to equal intensity with the aid of the wedge, which for this purpose is provided with a precision vernier (V).

To reduce interferences of phosphorescence, induced mainly by blue actinic illumination, the reaction vessel and the lenses surrounding it were made of quartz.

This compact arrangement with lenses of high aperture allows for optimal collection of the detecting light, which is severely scattered by samples of biological material. The short pathlength through the sample (5 to 10 mm) may be disadvantageous for work with dilute pigment solutions in vitro, but this aspect is immaterial for biological samples, in which the natural pigmentation of the individual chloroplasts is predetermined and generally dense.

One of the 2 rotating discs (D_s) (ca. 500 rpm), located close behind LL_2), serves to transmit alternately beams W and AB onto the photocathode P_m, so that their respective intensities can be compared via a properly arranged electronic detecting system. Synchronous switching of this system was performed with the aid of a 3rd small disc (D_t), mounted on the same axle as D_s (cf. below). The 2nd rotating disc (D_f, closest to the photocell in figure 1) serves to illuminate the sample with the actinic beam in a phosphoroscope arrangement. It is provided with 2 slits, opposed to 2 projections. The slits serve to transmit the actinic beam (twice per revolution), whereas the projections at the same moments blank out the photocell (in order that it not be affected by stray and fluorescent light induced by the flash). Disc D_f spins 4 times faster (ca. 2000 rpm) than the slow disc (D_s), thus yielding a much improved flash shape and—compared to our earlier single disc arrangement—considerably reduces the time-lapse between the flash and the reillumination of the photocathode by the detecting beam. The actinic light source is focussed on a fixed slit, close to D_f, with the aid of a condensing lens. A train of lenses and a mirror concentrate its light on a ca. 5 × 5 mm area of the sample cuvette. A shutter (s) and holders for color or wire gauze filters (FF) are provided in the light path.

The only limitation in the choice of the actinic light source is the hazard of phosphorescence induced, mainly by short wave length light, even in quartz. Interference by these effects, as well as of algal chemiluminiscence, in most cases can be avoided by using strong enough detecting light or, if necessary, cor-

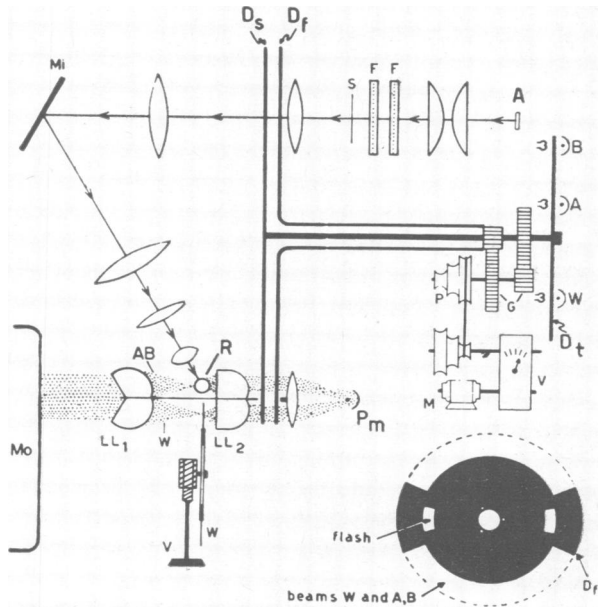


Fig. 1. Schematic illustration of the split-beam apparatus. See text for details. For clarity, baffles, slits, etc., arranged to prevent actinic illumination from reaching the photocell, are not shown. To the lower right the fast disc D_f is shown.

rected for (10). For photosynthesis experiments, incandescent lamps or Xenon arcs are best suited.

A photomultiplier is used as the light detector, since fast time response (10^{-5} sec rise time) and, in several instances, also high sensitivity are required. The signal, generated by the photocell, consists of a series of square pulses—2 per turn of the fast disc, i.e., 8 per turn of the slow disc (cf. fig 3). It is fed into a D.C. amplifier (cf. figure 2), where it is clipped so that only the top part of the pulses—which contain the desired information—appear at the output. The output is fed into 3 identical channels, which can be opened and closed by electronic switches. These switches are operated via photocells actuated by the 3rd rotating disc (D_t, cf. figures 1 and 3). As long as a cut-out in disc (D_t) is in front of a photocell, the corresponding channel is open and the momentary value of the photo-signal is transmitted and stored via an integrating network. For reasons mentioned earlier (11), the primary photo-signal should be kept constant (V_1 in fig 2) in each series of observations. For this purpose the output of one of the channels is amplified and regulates the photomultiplier voltage (V_2, fig 2). In addition this arrangement automatically compensates for slow drifts of the amplifier and of the detecting beam.

ARRANGEMENTS WITH SHORT OR LONG DARK TIMES: Two types of measurement have so far been performed with the described set up:

Short dark times: For this type of experiment a slow disc, shaped as shown in figure 3 (D_s top left), was used. Beams AB and W were alternated during equal time periods (8 × per revolution). Synchronously, disc D_t switched from 1 amplifier channel to the other (only 2 channels are needed in this case). Exactly at the moment that disc D_s switched from 1 beam to the other, a separate hole in it coincided with a cut-out in the fast disc—so that a flash hit the sample (and the photocell was briefly darkened). This course of events is illustrated in figure 3 (top right), in which, for clarity of illustration, a large inequality between the signals AB and W is drawn. The time distance between the flashes is about 12 milliseconds, presumably too short to allow the dark reactions to remove much of the photoproducts. These, therefore, will pile up during a sequence of flashes and attain a steady state concentration, close to the level prevailing in strong continuous light. Changes of absorption, eventually induced by such a virtually continuous illumination, now can be detected, if before adding the flashing light, beams AB and W are equalized (with the aid of the wedge W, fig 1), so that the difference recorder yields zero deflection.

Long dark periods: By exchanging the discs D_s and D_t, discussed above for the ones shown in the bottom part of figure 3, a different type of measurement, viz. with flashing light with "long" dark periods, as used in our earlier work, could be performed. Since disc D_s in this case is provided with only 2 holes for transmitting the actinic beam, a flash occurs only 1 out of 4 times a slit in the fast disc passes by. At 500 rpm of D_s, such a coincidence occurs only once per 60 milliseconds. This gives a dark time long enough to allow a considerable decay of short lived photoproducts, even under conditions of steady state flashing light.

The transmission of the sample is observed immediately before the flash (observation B) and again immediately after it (observation A). The desired pattern of observation times is selected by disc D_t and illustrated by the dotted areas in figure 3, bottom right.

During each of the long dark periods there is ample time for making a 3rd observation; namely, of the reference beam W, which is transmitted by disc D_s during half the cycle (one quarter revolution). The trigger disc is provided with only 1 cut-out per cycle (equal to one half revolution), which at the proper moments opens the amplifier channels denoted B, A and W.

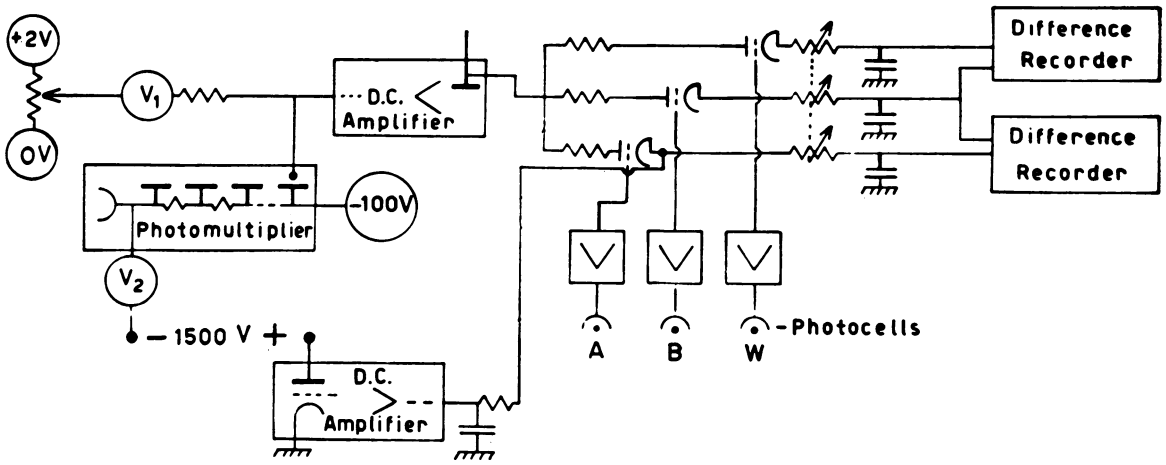
The diagram in figure 3 shows that during each cycle the photomultiplier is shut off 4 times instead of once by the fast disc. This is immaterial, since the 3 observations are made in between these closures.

The arrangement with long dark periods allows, firstly, to measure reversible (or step-wise) absorption changes by comparing signals B and A—at all times equal, if no actinic illumination is given.

Secondly, a comparison between signals W and B yields information concerning the absolute absorbance level at the end of the dark periods and thus indicates the density background (eventually also changed in the light), on which "fast" changes, A minus B, are superimposed.

By repositioning the 3 triggers, one can, if desired, also measure, for instance, A−W and B−W simultaneously. However, the 3 channels and the 2 recorders are closely alike, so that quantitatively (B−W) + (A−B) = (A−W) and 2 measurements are sufficient.

Measurement A−B concerns such fast changes that, compared to the duration of the individual effects, the integration and the response time of the recorder is inherently slow and therefore rather immaterial in any aspect other than accuracy. But in several instances measurement B−W (and also AB−W in the 2 channel set-up) indicates background absorption changes with time courses of the order of seconds. To study these, a rather fast response of the apparatus (down to 1 second) is advantageous. In this case such a fast indication is unobjectionable since the sensitivity of the method is mainly limited by differential fluctuations between the 2 beams (the V_2 compensating device, of course, can sense only 1 of the channels). Such fluctuations are not statistically random and little is gained by prolonged integration periods. (This is in contrast to the A−B measurements made with a single beam, which gain considerably in accuracy by prolonged integration).



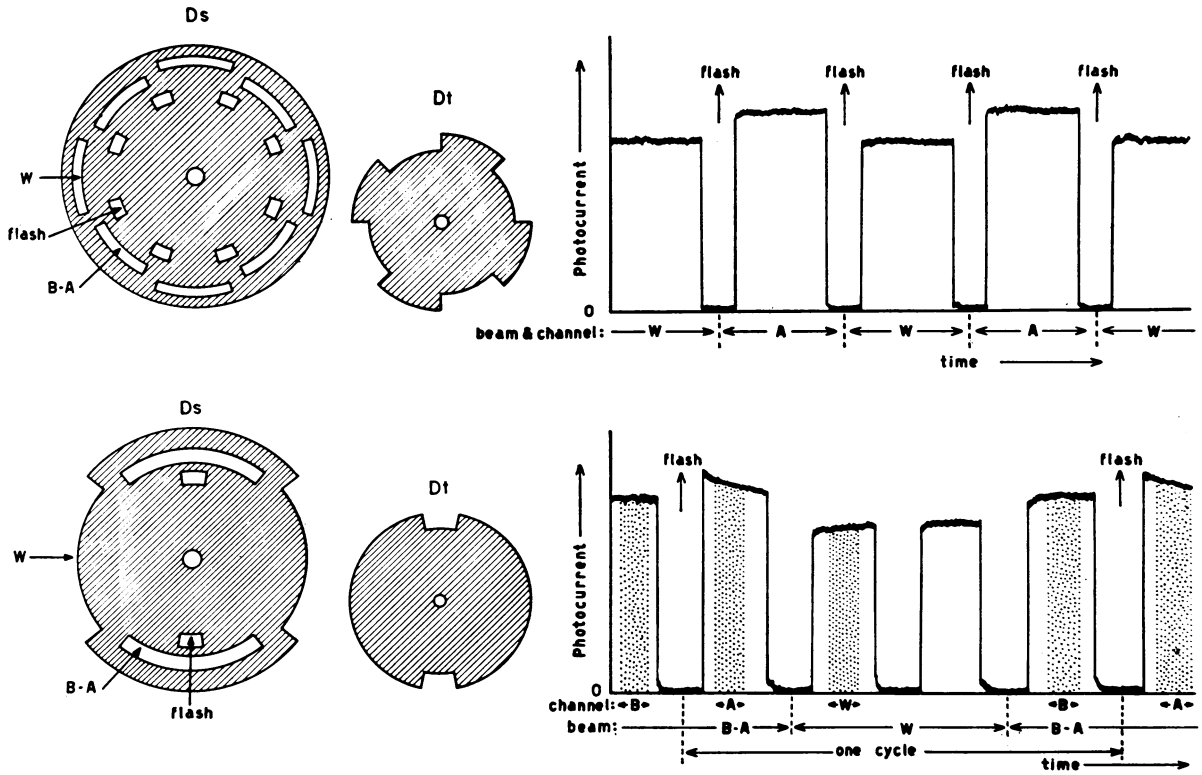


FIG. 2 (*top*). Simplified arrangement for the amplification, selection and recording of the photomultiplier signals. See text for discussion.

FIG. 3 (*bottom*). Illustration of the time courses of photocurrent (right) obtained with the "short dark time" arrangement (top) and the "long dark time" arrangement (bottom). The shapes of the slow disc (D_s) and the trigger disc (D_t) are also illustrated for each set-up (left).

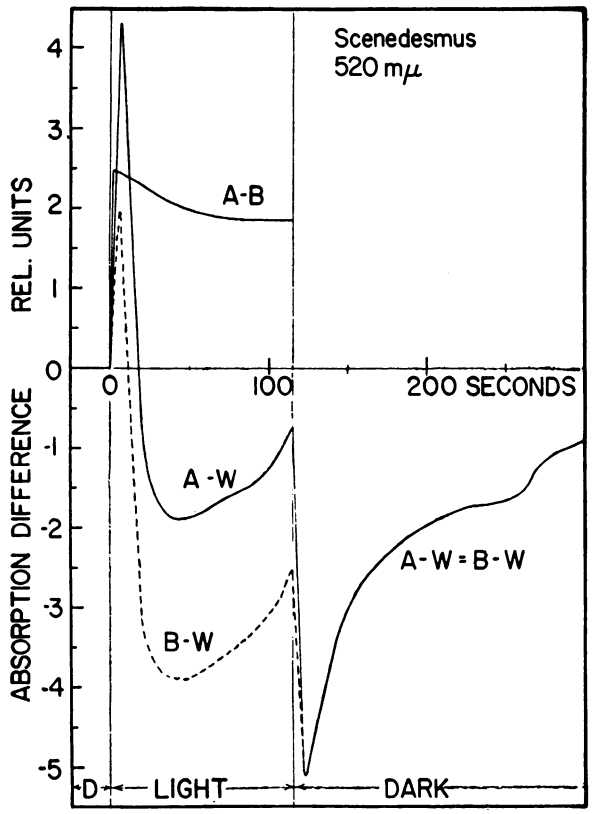


FIG. 4. Time course of signals A—B, B—W and A—W, as measured in 2 parallel experiments differing only in respect to the positioning of the trigger photocells (signal A—W therefore was directly measured rather than computed as the difference between the 2 other signals). Scenedesmus, I_o = 520 mμ. White actinic light.

EXAMPLES OF APPLICATION: As already mentioned, our main purpose for the described apparatus was a more intensive study of phenomena occurring in the red wave length area. Most likely, these bear closely on the primary photosynthetic events. Few investigations concerning these events have been made so far and these led to either negative or inconsistent results (1, 3, 11, 12, 14). Our first experiments with the apparatus described were made with the short dark time arrangement. The effects obtained did not agree with our earlier published data concerning fast effects. Subsequently therefore, mainly the 3 channel, long dark period method was used in studies of simultaneous rapid reversible effects and background changes.

The data of figure 4 illustrate that the complexity of the effects encountered, rather than methodological pitfalls often are responsible for apparently incongruent results. These data were chosen because they concern the extensively studied effects occurring around 520 mμ in Scenedesmus (3, 11, 12, 14, 18). The peculiar time course of the background curve (B—W), i.e., the absorbance level some 0.06 seconds after each light flash, closely matches data obtained by Strehler and Lynch (4) using a different method. On the other hand, in accord with our own earlier findings, the magnitude of the fast changes (A—B) is entirely unaffected by these seemingly drastic changes of background absorption (in fact all effects are within a range of only a fraction of a percent transmission change). Apparently the individual light flashes produce a constant amount of the molecular species involved. During the 1st 10 seconds of illumination, its formation is faster than its removal during the dark periods. After this induction period the rate of removal in the dark increases and temporarily surpasses the rate of formation so that at any moment of the flashing light cycle absorption is lower than the dark level. In contrast, our short dark time method showed—in accordance with other observations made in continuous actinic light—an increase of absorption in this wave length region.

Another peculiar phenomenon—of which one should be constantly aware in this type of measurement—is demonstrated in figure 5. The data, obtained with *Rhodospirillum rubrum*, show that at both wave lengths studied, a decrease of the intensity of the detecting beam (I_d) considerably increases the magnitude of the background absorption change (B—W) induced by the flashes. A 3-fold difference is observed in these particular examples. In other cases, we have observed both larger and smaller antagonistic effects between actinic and detecting lights. In the next section we will mention an observation of the reversed effect, i.e., the requirement for strong detecting light to obtain significant actinic light action.

Noteworthy in figure 5 is the observation that the magnitude of the fast changes (A—B which appears superimposed on B—W in curve A—W) is unaffected by the intensity of I_d. Also the fact that deflection B—W is at least equal to deflection BA appears significant (cf. below).

Figure 6 gives another example of difference spectra, each set of which was measured in 2 experimental runs with parallel samples of bacteria: 1st A—B together with B—W, 2nd A—B concurrently with A—W. The particular curves measured with *Rhodospirillum rubrum* do not show any special effects and are in general accord with Duysen's data obtained with continuous illumination (2). The 3 different curves follow a closely similar pattern, which indicate that the flash-induced changes revert in the dark, but not completely. Obviously, in the steady state flashing light pattern a considerable background concentration of converted pigment persists. Actually, as will be described in a later paper, the same situation seems to hold for nearly all the absorption shifts, which occur so abundantly over the range between 400 and 850 mμ in purple sulfur bacteria.

An interesting phenomenon in figure 6 can be observed in the region around 885 mμ in the spectra measured with Chromatium. Here both the A—B and A—W curves show a distinct band of absorption decrease, which, however, is absent in the B—W

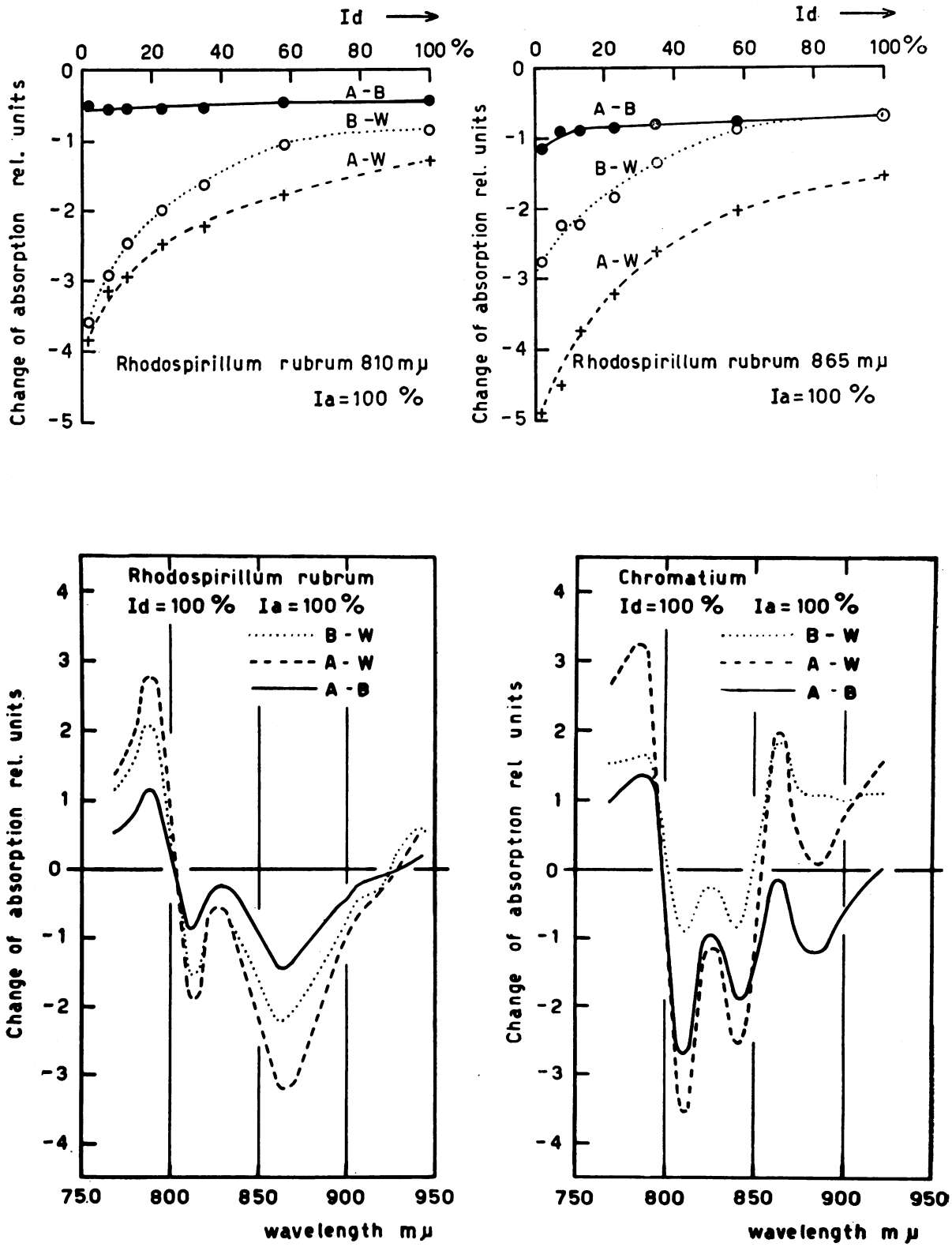


FIG. 5 (*top*). Dependence of the magnitude of signals A—B, B—W and A—W (all 3 measured in 2 experimental runs) upon the relative intensity of the detecting beam.

FIG. 6 (*bottom*). Difference spectra as measured in the infra-red with purple sulfur bacteria. Actinic light: 500 to 700 mμ obtained from a filtered mercury arc.

spectrum. This and related observations in photosynthetic bacteria seem to show basic similarities to the phenomena touched upon in the next section.

A RED, FAR-RED ANTAGONISM IN PHOTOSYNTHESIS: Partly to illustrate the versatility of the apparatus we may briefly describe a few observations made with the blue green alga, Anacystis. This alga was chosen as a subject of study because of its ready growth, its small size, which makes settling in the sample tube less of a problem, and because in our earlier work (11) blue green algae showed a quite pronounced reversible decrease of absorption around 705 mμ.

The phenomena observable in the red part of the spectrum appear to be extremely complex and the data to be described represent only the first steps towards a complete analysis.

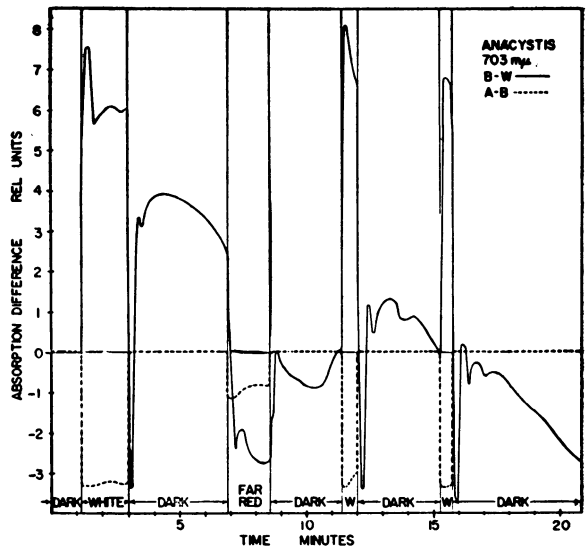
We will restrict ourselves to some observations made in the wave length region between 690 and 720 mμ. Ribbon filament (6 V, 18 A) incandescent lamps were used both for the actinic and the detecting beam, a 1 cm water filter containing a trace of copper sulfate was at all times in the actinic beam.

Figure 7 shows a time course of the difference B−W measured with I_d = 703 mμ by alternately illuminating the sample with either white (non-filtered incandescent) or far-red light. A Schott RG 1 ($\lambda > 600$ mμ) filter in the actinic beam hardly decreased (↘ 30 %) the white light effect. Far-red light was obtained by placing a Schott RG 5 ($\lambda > 670$ mμ) in the beam; a Schott RG 8 or filter ($\lambda > 695$ mμ) yielded some 40 % smaller deflections.

The data in figure 7 indicate that far-red light yields a decrease of background absorption whereas red or white light has the reverse effect. The white or red light actually contained at least as much far-red radiation as was transmitted by the RG 5 filter. Obviously therefore the effectiveness of the 600 to 670 mμ radiation is predominant in this mixture. Also shown in this figure (dashed curve) are the deflections A−B caused by the respective illuminations. Note that these are negative, regardless of color, only the magnitude varying. The A−B deflections (fast reversible changes) are smaller than the B−W changes. So far we have often been able to observe exact equality of the 2 deflections and when unequal, A−B never exceeded B−W (disregarding signs of the changes).

In the case that deflection B−W is exactly equal and opposite to deflection A−B, (illustrated in fig 9), we meet the interesting case that the flashes destroy exactly as much "700 pigment" as is built up again in the dark period (A−W = 0). This pigment (or at least this active fraction) thus might not be present in the dark before the actinic illumination, neither may it become apparent under steady state illumination.

In this type of experiment the white light intensity required for building up the 700 pigment is quite low. Our earlier work showed that saturation of the fast effects (A—B) required intensities of the order of



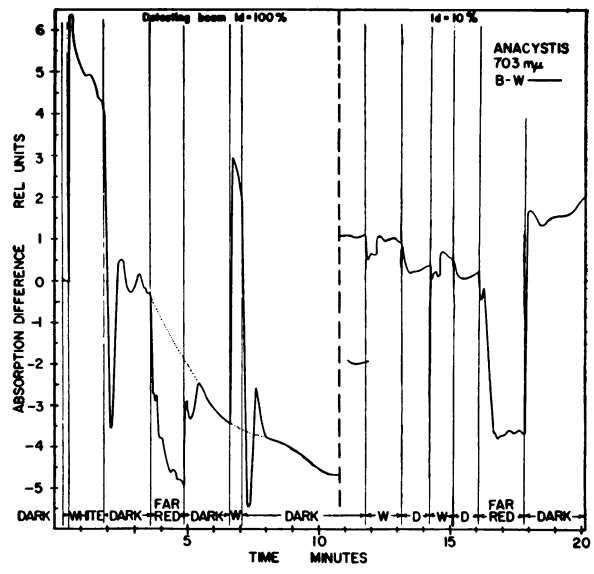


FIG. 7 (*top*). Time course of signals B—W (solid line) and A—B (dashed line) as observed with Anacystis cells alternately exposed to either white or far-red flashing light. Note the occurrence of transitory phenomena in many instance, and of slow absorption decay in the "dark" induced by white illumination. The full intensity of the detecting beam was used. The A—B deflections are negative in all instances.

FIG. 8 (*bottom*). Left: Similar experimentation as described in the legend of figure 7 except for omission of signal A—B. Right: Continuation of the experiment after the detecting beam was decreased to 10 % of the original value: effects in white light tend to disappear.

those needed for photosynthetic flash-saturation. But half-saturation of the background increase was already obtained at intensities some 3 to 10% of the available actinic light. Such intensities yield barely observable fast (A—B) effects. Even 100 % of the used incandescent illumination is rather weak in terms of photosynthetic activity. We tend to ascribe this low intensity requirement to the fact that a counteraction between detecting and actinic beam is involved. The detecting beam in itself (cf. e.g., figs 5, 7, 8) is strong enough to evoke considerable effects, observable as strong drifts of absorption, dependent in sign, magnitude and duration upon wave length setting and prehistory of the sample.

On the other hand, as is illustrated in figure 8, we also found that in extremely weak 705 mμ detecting light, the (B—W) red effect may disappear completely, whereas the far-red effect may be either unaffected or considerably increased. Apparently, since beam I_d does not cause appreciable breakdown, red light cannot further increase the concentration of 700 pigment, but far-red light finds abundant material to convert. However, since the A—B changes are hardly affected by a decrease of I_d, we obtain the reverse situation as was met before: instead of A—W being zero and B—W positive, now B—W is zero and A—W is negative. Generally, the relative background concentration of the 700 pigment indicated by signal B—W will tend to attain an intermediate steady state level, determined by the prevailing conditions, often preceded by transitory adjustments.

Figure 9 finally illustrates the close similarity of peak configuration—regardless of sign—of the 3 types of difference spectra.

The data discussed are far from exhaustive and our reasoning is necessarily over-simplified. The mere fact that a light flash photo-chemically destroys the pigment, but at the same time induces its reformation in the following dark period, indicates that dark phenomena (and therefore e.g., temperature effects) must be involved.

The described type of mechanism shows obvious similarities to the light effects encountered in plant growth regulation and might well be of more general significance in photochemical conversion by plants (7, 16, 17).

A significant interpretation of the described phenomena can be given only because of fundamental discoveries of Emerson and co-workers. In 1941, Emerson and Lewis (9) found in Chlorella a severe drop of the photosynthetic quantum yield at wave lengths beyond 680 mμ. This same phenomenon was observed in red and blue green algae (4, 8) as well as in Diatoms (11). Later, an influence of temperature upon the yield and long wave length-limit of photosynthesis was observed. The most striking observation, however, was that this long wave length-limit could be shifted towards the far-red by the addition of a supplementary amount of short wave length light ($\lambda < 650$ mμ). The conclusion from these data was that excitation of chlorophyll a alone—

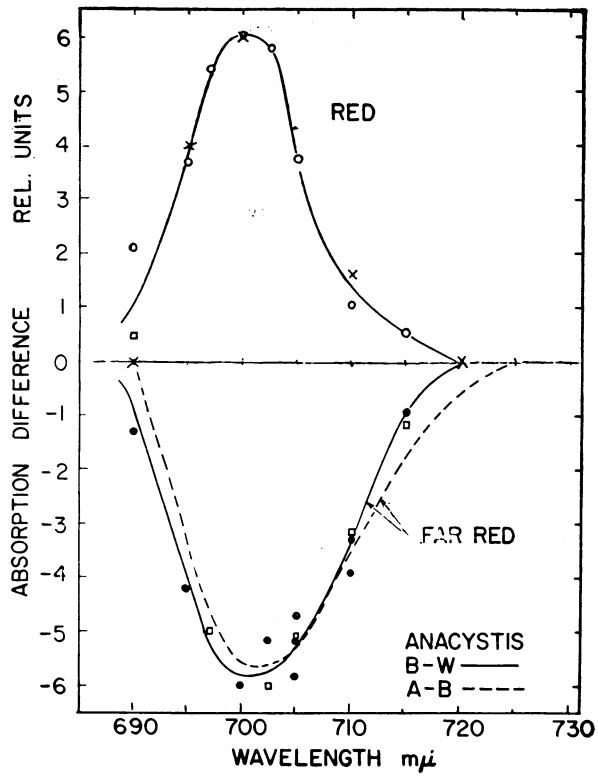


FIG. 9. Difference spectra B—W as induced by red light (incandescent with RG1 filter, open circles, crosses) and by far-red light (incandescent with RG5 filter, dots, squares). The dashed curve shows the A—B spectrum which was measured concurrently with the B—W observations indicated as dots. Curves are adjusted for equality of peak height.

although the only universally occurring photosynthetic pigment—does not yield efficient photosynthesis (6, 7).

In the light of Emerson's findings we can at least tentatively try to explain some of the effects we have observed. The location of the absorption band of the 700 mμ pigment—even if present in only a very small concentration—makes it ideally suited to effectively trap all the light which is absorbed by chlorophyll a itself, or transferred to it by accessory pigments (and which thus is transformed into "far-red" light). Suppose the 700 pigment is indeed the final light sink in photosynthesis and its excitation by surrounding chlorophyll a molecules leads to a conversion, which entails disappearance of its absorption. Then, from there on, the neighboring chlorophyll a molecules—say those comprising a "unit"—are deprived of their outlet until the 700 pigment is restored. This restoration, however, also requires light, but now only red and not far-red is active. Obviously, sensitization by chlorophyll a (which yields only 700 mμ fluorescence) cannot restore the bleached 700 pigment and photosynthesis cannot proceed effectively in far-red light alone.

As was described above, irradiation with red light restores the 700 mμ pigment and therefore sustains the conversion of irradiation transferred to it via chlorophyll a.

Further speculation, however tempting, does not appear to be fruitful at the present time. We would even have hesitated to presently venture the above thoughts if it were not to draw attention to a new road towards a better understanding of the most fundamental aspects of photosynthesis, opened up by the eminent scientist to whose memory this article is dedicated.

Summary

An apparatus is described, designed to register the small changes of absorption, which are induced by light in photosynthetic organisms. A split-beam arrangement allows the simultaneous observation of fast reversible absorption changes, caused by flashing actinic illumination and of slow shifts of background absorption.

A few examples of its application are discussed. One of these concerns the observation of an antagonistic effect between red and far-red light on Anacystis. It concerns the build up and break down of a pigment, normally absorbing at 700 mμ, present in low concentration, and tentatively identified as the end point of energy transfer in photosynthesis.

References

1. Coleman, J. W., Holt, A. S. and Rabinowitch, E. I. Reversible bleaching of chlorophyll in vivo. Research in Photosynthesis, H. Gaffron, ed. Pp. 68–74. Interscience, New York 1957.
2. Duysens, L. N. M. Transfer of excitation energy in photosynthesis. Thesis, Utrecht 1952.
3. Duysens, L. N. M. Methods for measurement and analysis of changes in light absorption occurring upon illumination of photosynthesizing organisms. In: Research in Photosynthesis, H. Gaffron, ed. Pp. 59–67. Interscience, New York 1957.
4. Emerson, R. and Lewis, C. M. The photosynthetic efficiency of phycocyanin in Chroococcus and the problem of carotenoids participation in photosynthesis. Jour. Gen. Physiol. 25: 579–596. 1942.
5. Emerson, R. and Lewis, C. M. The dependence of the quantum yield of Chlorella photosynthesis on wave length of light. Amer. Jour. Bot. 30: 165–178. 1943.
6. Emerson, R., Chalmers, R. and Cedarstrand, C. Some factors influencing the long-wave limit of photosynthesis. Proc. Natl. Acad. Sci., U.S. 43: 133–143. 1957.
7. Emerson, R. The quantum yield of photosynthesis. Ann. Rev. Plant Physiol. 9: 1–24. 1958.
8. Haxo, F. T. and Blinks, L. R. Photosynthetic action spectra of marine algae. Jour. Gen. Physiol. 33: 389–422. 1950.
9. Kok, B. On the reversible absorption change at 705 mμ in photosynthetic organisms. Biochim. Biophys. Acta. 22: 399–401. 1956.
10. Kok, B. Absorption changes induced by the photochemical reaction of photosynthesis. Nature 179: 583–584. 1957.
11. Kok, B. Light induced absorption changes in photosynthetic organisms. Acta. Bot. Néerl. 6: 316–336. 1957.
12. Kok, B. Changes of absorption spectrum induced by illumination and their bearing on the nature of the photoreceptor in photosynthesis. Proc. 2nd Inter. Congr. Photobiology, Turin. Pp. 369–383. 1957.
13. Kok, B. Photosynthesis in flashing light. Biochim. Biophys. Acta 21: 245–258. 1956.
14. Strehler, B. I. and Lynch, V. H. Studies on the primary process in photosynthesis II. Arch. Biochem. Biophys. 70: 527–546. 1957.
15. Tanada, T. The photosynthetic efficiency of carotenoid pigments in *Navicula minima*. Amer. Jour. Bot. 38: 276–283. 1951.
16. Toole, E. H., Hendricks, S. B., Borthwick, H. A. and Toole, V. K. Physiology of seed germination. Ann. Rev. Plant Physiol. 8: 299–324. 1957.
17. Wassink, E. C. and Stolwijk, J. A. J. Effects of light quality on plant growth. Ann. Rev. Plant Physiol. 8: 373–400. 1957.
18. Witt, H. T. Reaction patterns in the primary process of photosynthesis. In: Research in Photosynthesis, H. Gaffron, ed. Pp. 75–84. Interscience, New York 1957.