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# STUDIES ON THE ELECTRON-TRANSFER SYSTEMS IN PHOTOSYNTHETIC BACTERIA

## I. THE LIGHT-INDUCED ABSORPTION-SPECTRUM CHANGES AND THE EFFECT OF PHENYLMERCURIC ACETATE

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SUMMARY

Absorption-spectrum changes caused by illumination in photosynthetic purple bacteria, *Rhodopseudomonas spheroides* (wild type and blue-green mutant), *Rhodospirillum rubrum* (wild type and blue-green mutant) and *Chromatium*, were studied. Low-temperature spectrophotometry was advantageously used for the determination of heme components. Responses of the isolated chromatophores were compared with those of the intact cells.

Heme proteins were found to be functioning in the photosynthetic and respiratory electron-transfer system. The effect of several inhibitors, especially phenylmercuric acetate, was studied. Light-induced responses of different types of cytochromes were analyzed. Absorbancy changes corresponding to carotenoids and bacteriochlorophyll were observed in certain strains and preparations. Carotenoids showed evidence that they had some correlation with other electron-transfer catalysts. A light-induced change in chlorophyll was observed in *R. rubrum* chromatophores. There was no evidence of appreciable chlorophyll-absorbancy change in the intact cells under weak infrared illumination. A scheme for the electron-transport path in the photosynthetic and respiratory systems of photosynthetic bacteria was constructed from these data.

## INTRODUCTION

In the past few years there have been a number of investigations of absorption spectrum changes in photosynthetic bacteria upon illumination or introduction of oxygen. DUYSENS[1], and CHANCE AND SMITH[2] found the absorption spectrum changes in an anaerobic suspension of *Rhodospirillum rubrum* during low-intensity illumination. CHANCE AND SMITH discovered that the change was similar to that found after the addition of oxygen, and concluded that the illumination caused oxidation of more than one cytochrome in the bacterial cells.

Abbreviation: PMA, phenylmercuric acetate.

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OLSON AND CHANCE[3-5] studied the light-induced cytochrome oxidation in *Chromatium* strain D, another photosynthetic bacterium, and found that this oxidation, too, involved numerous cytochromes, as in *R. rubrum*. CHANCE[6] and SMITH AND RAMIREZ[7,8] observed the reversible absorption decrease, due to illumination or oxygenation, at the wavelengths corresponding to carotenoid peaks in the bacterial cells. SMITH AND BALTSCHEFFSKY[9] studied respiratory and light-induced phosphorylating systems in extracts of *R. rubrum*. They found the two systems different in a number of respects, and suggested a role of cytochrome $c_2$ in the light-induced phosphorylation.

We[10,11] have recently reported the temperature-independent oxidation of *c*-type cytochrome in *Chromatium* (down to 77° K) by bacteriochlorophyll-absorbed infra-red light, a phenomenon suggesting a mechanism for a non-thermal oxidation–reduction reaction between bacteriochlorophyll and cytochrome molecules.

The phenomenon of appearance in chromatophores or aerobic cells of a band around 430 mμ by illumination has been somewhat confusing. In *R. rubrum* cells suspended in aerobic water, DUYSENS[12] found an increase in absorption at 432 mμ by illumination. CHANCE *et al.*[2,13] had previously shown that PMA treatment caused cytochrome oxidation in the same species, and illumination after the treatment led to an appearance of a 430-mμ band. SMITH *et al.*[14] reported that illumination of a PMA-treated aerobic suspension of *R. rubrum* or *Rhodopseudomonas spheroides* induced an appearance of a broad absorption peak at 434 mμ. They also reported a similar phenomenon in *R. rubrum* extracts[9]. From our study it is concluded that two different phenomena have been confused. One seems to be the reduction of a cytochrome of *b*-type by illumination in PMA-treated *R. rubrum* or *R. spheroides* cells or washed aerobic *Chromatium* cells. The other is observed in *R. rubrum* chromatophores in aerobic and anaerobic states, and it is most probably an electronic change of an assimilatory pigment. These two phenomena will be discussed in detail (see DISCUSSION).

In this paper, further studies of light-induced absorption spectrum changes will be presented. A scheme for the electron transfer system will be presented and the roles of electron carriers will be discussed.

## METHODS

### *Culture of bacteria*

Except for wild type of *R. spheroides*, all bacteria were grown in a 25° water bath in full glass-stoppered bottles, illuminated by incandescent lamps of an approximate light intensity of 16000 lux. Fully-grown bacteria were used for the experiments. In many cases observations were made with suspensions taken directly from culture bottles. Cells were concentrated by centrifugation and resuspended in the original culture medium when needed. Usually *Chromatium* cells were harvested by centrifugation and resuspended in a fresh medium.

*R. rubrum*, van Niel strain 1 (obtained from Dr. M. D. Kamen) and a blue-green mutant of *R. rubrum*, strain G9 (obtained from Dr. J. W. NEWTON) were grown anaerobically in a medium described by GEST *et al.*[15] under illumination. *R. spheroides*, strain 241C, and its blue-green mutant, strain UV 33, were obtained from Dr. G. COHEN-BAZIRE and grown in her medium[16]. Unless otherwise stated, the blue-green mutant was grown anaerobically and the wild type *R. spheroides* semi-anaerobically

with illumination, to minimize the irreversible carotenoid changes caused by oxygen introduction[17]. *Chromatium* strain D, presented by Drs. M. D. KAMEN AND J. W NEWTON, was cultured in their medium[18].

*Preparation of R. rubrum and Chromatium chromatophores*

The method of preparation of chromatophores has been described in a previous paper[19].

*Spectrophotometric measurements*

Absorption spectrum changes by illumination, aerobiosis, etc., were measured by a split-beam spectrophotometer[20,21] and a double-beam spectrophotometer[22,23] developed in this laboratory. When necessary, a low-temperature apparatus[24] was modified to allow spectrophotometry of illuminated-minus-dark samples at liquid nitrogen temperature. An incandescent lamp and a Kodak Wratten 88A filter (removing light of wavelengths shorter than 720 mμ) furnished a near-infrared illumination for bacterial suspensions or chromatophores. A Corning 9788 filter protected the photomultiplier from the near-infrared illumination.

*Oxygen tension measurements in bacterial suspensions*

An oxygen electrode apparatus with a collodion-coated rotating micro-electrode[23,25] was occasionally used for determining oxygen consumption rate and ascertaining whether the bacterial suspension was aerobic or anaerobic.

## RESULTS

*R. spheroides wild type*

The pigments of this bacterium were clearly delineated by the absolute spectra at room and at low temperatures illustrated by Fig. 1. Here peaks due to bacteriochlorophyll (585 and 587 mμ, at room and at liquid nitrogen temperatures, respectively), and carotenoids (507; 509 mμ), (476; 475 mμ), (448; 447 mμ) can be identified.

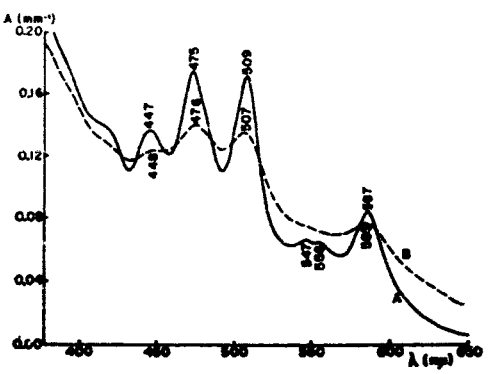


Fig. 1 Absorption spectra of anaerobic suspension of *R. spheroides* cells, at 77° K (A) and 299° K (B). Optical path length, 1 mm; 0.54 mg dry weight/ml.

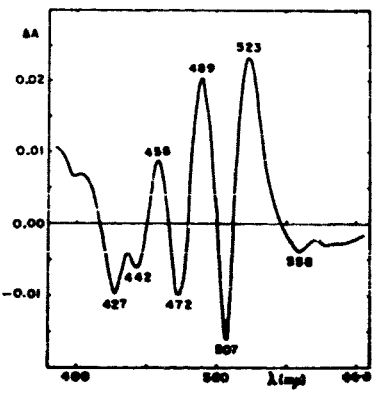


Fig. 2. Illuminated minus dark difference spectrum of anaerobic suspension of *R. spheroides* cells. Optical path length, 3 mm; 2.11 mg dry weight/ml; temperature, 299° K.

In addition, the low temperature spectrum also indicates two peaks at 556 mμ and 547 mμ which are presumably the α-bands of cytochromes. As has been pointed out in several publications[6–8], these carotenoid bands are affected by illumination (Fig. 2). It is apparent that the 507-mμ peak was replaced by one at 523 mμ; a similar shift to a longer wavelength occurred with the peak observed, in the absolute spectrum at 476 mμ but at 472 mμ in the difference spectrum. A similar change may have affected the band at 448 mμ to give a band shift from 442 mμ to 458 mμ in the difference spectrum. The apparent shift by illumination to the longer wavelength in the difference spectrum was 16–17 mμ. The peak at 427 mμ in Fig. 2 is no doubt due to cytochrome oxidation as will be discussed later. Potassium cyanide ($1 \cdot 10^{-3}$ *M*) did not inhibit the light-induced cytochrome oxidation or the carotenoid absorption band shift in anaerobic suspension of *R. spheroides*.

The response of the carotenoids to illumination was more clearly shown if they were treated with PMA ($9 \cdot 10^{-5}$ *M*) (Fig. 3). In this case, the shift of the 448 mμ carotenoid band in the absolute spectrum (Fig. 1) (445 mμ in the difference spectrum, Fig. 3) to 458 mμ was clearly defined and the shifts towards longer wavelengths, for the three peaks, were 13, 16 and 16 mμ, respectively.

It has been pointed out that a treatment of *R. rubrum* with PMA causes the light-induced cytochrome oxidation to disappear and to be replaced by a large absorbancy increase at about 430 mμ (see refs. 2, 13). This effect was more clearly revealed in *R. spheroides*. Fig. 3 shows the appearance of a rather sharp peak having a maximum at 429 mμ. Like a band observed in *R. rubrum* (Fig. 10), this sharp band observed in *R. spheroides* is strongly suggestive of heme protein reduction. It is tentatively interpreted as reduction of a *b*-type cytochrome by illumination, when all the cytochrome components remain in the oxidized state in the presence of the inhibitor. This change is similar to the change observed when oxygen is introduced to anaerobic cells (in the opposite direction)[17].

A study of the effect of different concentrations of PMA for producing spectral changes of the type indicated in Fig. 3 is shown in Fig. 4. At a PMA concentration

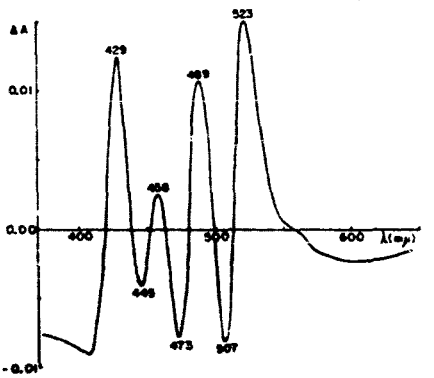


Fig. 3. Light-induced change in aerobic cells of *R. spheroides* in the presence of PMA ($9 \cdot 10^{-5}$ *M*). Illuminated minus dark difference spectrum. 0.434 mg dry weight/ml. 10 mm optical path. Temperature, 296° K.

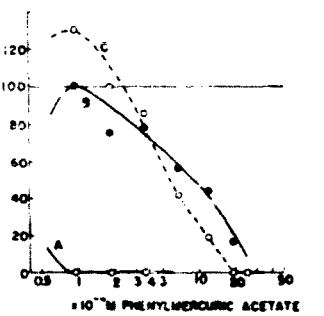


Fig. 4. PMA inhibition of light-induced spectrum changes in *R. spheroides* cells. A, disappearance of 427-mμ band; B, appearance of 429-mμ band in the presence of PMA; C, disappearance of 507-mμ band. Ordinate indicates relative magnitude of changes.

of $1 \cdot 10^{-4}$ *M*, a large 429-m$\mu$ band was observed under illumination. Higher concentrations inhibited the appearance of the 429 m$\mu$ band and the disappearance of the 507-m$\mu$ band (carotenoid shift). This result is rather different from that observed in *R. rubrum* where a wide range of PMA concentrations could be employed, and the result suggests a higher SH-group sensitivity in the basic light response of *R. spheroides* than that of *R. rubrum*.

No effect of carbon monoxide on the light-induced absorption spectrum change in the PMA-treated aerobic cells was observed. This suggests the existence of a cytochrome of *b*-type besides a CO-binding pigment, RHP/cytochrome *o* (see ref. 17). It is also indicated that the CO-binding pigment is not located between the cytochrome *b* and the photochemical reducing site. Illuminated minus dark difference spectra of the PMA-treated cells with and without carbon monoxide are shown in Fig. 5.

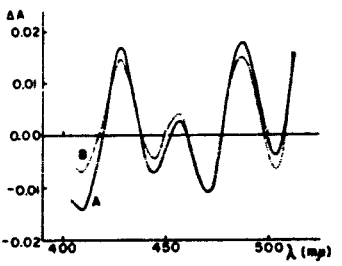


Fig. 5. Effect of CO on light-induced absorption spectrum change in PMA-treated cells of *R. spheroides*. Aerobic cells, 0.99 mg dry weight/ml; [PMA], $1 \cdot 10^{-4}$ *M*; pure CO saturated, optical path length, 10 mm; temperature, 297° K. A, illuminated minus dark, PMA added; B, illuminated minus dark, PMA and CO added.

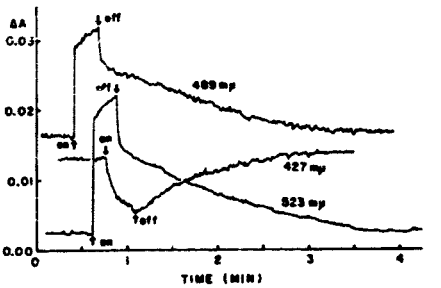


Fig. 6. Kinetics of light-induced absorption spectrum changes at three different wavelengths, 427 m$\mu$, 489 m$\mu$ and 523 m$\mu$. Anaerobic suspension of *R. spheroides* at 296° K, 1.44 mg dry weight/ml; optical path length, 10 mm.

In Fig. 6 the time course is plotted of the absorbancy changes observed upon initiation and cessation of illumination of suspension of *R. spheroides*. Two carotenoid shifts (489 m$\mu$ and 523 m$\mu$) and one cytochrome (427 m$\mu$) were monitored. As has been pointed out by SMITH AND RAMÍREZ[8], the carotenoid shifts appeared more rapidly than the cytochrome change. Oxygenation of the anaerobic suspension caused a similar shift of the absorption bands and a cytochrome oxidation. Here, too, the carotenoid shift was observed to be larger compared with the cytochrome change. It has been reported by CHANCE[6] that the rate of the carotenoid change was larger than that of the cytochrome change in the rapid oxygenation experiments. The more rapid response of the carotenoids at room temperature is a remarkable phenomenon since it has been observed in *Chromatium* that the quantum requirement for cytochrome oxidation is roughly two per electron[5]. If this value applies to *R. spheroides*, and if one further assumes that the carotenoid extinction coefficient is no greater than that for cytochrome, we would conclude that the quantum requirement for the carotenoid shift is indeed small.

*Blue-green mutant of R. spheroides*

With illumination or introduction of oxygen, the blue-green mutant of *R. spheroides* gave results similar to those obtained with the blue-green mutant of *R. rubrum*, which will be described in this paper and a succeeding paper[17]. Results with the blue-green mutant of *R. spheroides* were essentially the same as those of SMITH AND RAMÍREZ[7].

*R. rubrum wild type*

*R. rubrum* is probably the most extensively investigated of the purple bacteria and it was upon this organism that both DUYSENS[1] and CHANCE AND SMITH[2] made their original observations of the response of a single cytochrome (DUYSENS) and a number of cytochromes (CHANCE AND SMITH) in anaerobic cells. Interestingly enough these cells have proved to be the most difficult to work with. The superposition of cytochrome and carotenoid bands are such that the α-band cannot be adequately delineated, as was pointed out in the communication of CHANCE AND SMITH[2]. An example of the complex mixed spectrum of carotenoid and cytochrome changes by illumination is presented in Fig. 7.

Quinacrine hydrochloride ($1 \cdot 10^{-4} M$) or potassium cyanide ($6.5 \cdot 10^{-4} M$) had little effect on the light-induced absorption spectrum change (disappearance of a 428-mμ band) in anaerobic *R. rubrum* cells. Carbon monoxide did not inhibit the anaerobic light-induced change. The kinetics of the light-induced change in an anaerobic suspension of *R. rubrum* in the presence of carbon monoxide was the same as in untreated cells.

It has been known for some time that the kinetics of absorbancy change at the cessation of illumination of *R. rubrum* is diphasic in the greater part of the spectrum.

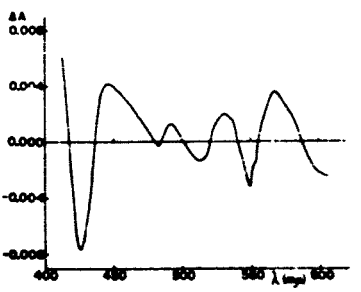


Fig. 7. Illuminated minus dark difference spectrum of anaerobic suspension of *R. rubrum* cells. Optical path length, 10 mm; 1.54 mg dry weight/ml; temperature, 296° K.

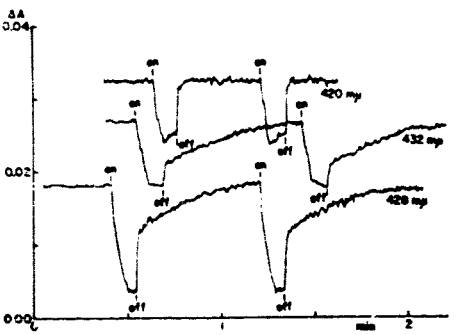


Fig. 8. Kinetics of absorption spectrum changes by illumination in anaerobic *R. rubrum* cells. Split-beam spectrophotometer recordings at 420 mμ, 428 mμ and 432 mμ. Optical path length, 10 mm; 2.16 mg dry weight/ml; temperature, 296° K.

A typical example is presented in Fig. 8. As is clear from the figure, at 428 mμ, there were large rapid and slow phases, but at 432 mμ, the rapid phase was small; at 420 mμ, decay was very rapid and there was no slow phase. This phenomenon has been described in considerable detail in studies of *Chromatium*[4] and a preliminary study has

been made in *R. rubrum*[6]. A more detailed study of the fast and slow absorbancy changes at the cessation of illumination in anaerobic *R. rubrum* is indicated in Fig. 9. The figure shows that a pigment absorbing in the region near 425 mμ was reduced much more rapidly at the cessation of illumination than that with a peak at approximately 430 mμ. This would lead to the conclusion that a pigment of the *c*-type predominates in the rapid response to the cessation of illumination while a pigment of the *b*-type predominates in the slow response. OLSON AND CHANCE[4] postulated four cytochrome species on the basis of kinetics and spectral characteristics of absorbancy changes of *Chromatium* observed under various conditions. Of the four cytochromes ($c_{422}$, $c_{430}$, $c_{423.5}$ and $c_{426}$), $c_{422}$ and $c_{430}$ were assumed to be reduced in the light-off transition under anaerobic conditions. These possibly correspond to the two components observed in the light-off absorbancy change in anaerobic *R. rubrum* cells.

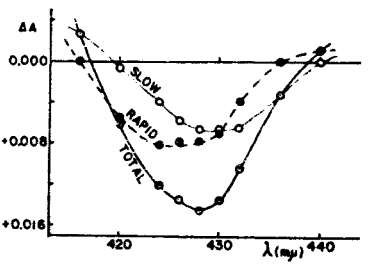


Fig. 9. Rapid and slow phases of light-off reaction in anaerobic *R. rubrum* cells. Optical path length, 10 mm; 2.16 mg dry weight/ml; temperature, 296° K.

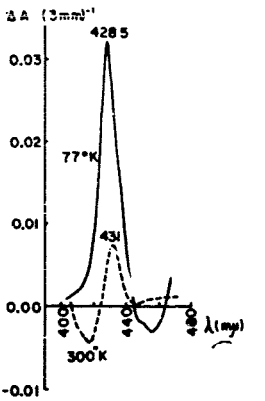


Fig. 10. Illuminated minus dark difference spectrum of PMA-treated *R. rubrum* cells. [PMA], $5 \cdot 10^{-4}$ *M*; optical path length, 3 mm; temperature, 77° K and 300° K.

It has already been mentioned that the absorbancy change caused by illumination of the PMA-treated *R. rubrum* cells has been observed in such a highly light-absorbing suspension that it was difficult positively to identify it to be a heme protein reduction. The width of the band seemed to vary with different experimental conditions: a narrow peak was recorded by CHANCE AND SMITH[2], while SMITH, BALTSCHEFFSKY AND OLSON[14] recorded a rather broad peak. In Fig. 10 the dashed curve represents a recording at room temperature of the illumination-induced change of the PMA-treated *R. rubrum* cells and it is seen that a peak with a maximum at 431 mμ appeared. If this illuminated material was chilled to the temperature of liquid nitrogen and the spectrum again recorded, it was seen that the peak was shifted to 428.5 mμ and had a half width of 15 mμ, a value quite in accord with the values observed for heme proteins under these conditions[24,27,28]. It was further found that the absorbancy change of Fig. 10 did not occur if the cells were treated with an excess of dithionite. No effect of carbon monoxide upon the light-induced absorption change in the PMA-treated cells (appearance of 431 mμ band) was observed. These data suggest the reduction of a *b*-type cytochrome by illumination in the PMA-treated *R. rubrum* cells.

*Blue-green mutant of R. rubrum*

Through the kindness of Dr. J. W. NEWTON we have obtained a culture of this valuable mutant and have been able to make more incisive studies of the cytochromes of *R. rubrum*. Fig. 11 represents absolute spectra of the anaerobic cells at room temperature and liquid nitrogen temperature. At room temperature two bacteriochlorophyll bands were observed at 374 mμ and 587 mμ. At liquid nitrogen temperature the chlorophyll bands were observed at the same positions and of considerably higher intensity. At the lower temperature, trace C of Fig. 11 shows a peak and a shoulder indicating cytochrome bands in the α-band region. A comparison of this figure with Fig. 1 indicates clearly the complete absence of spectroscopically detectable carotenoids in the mutant cells.

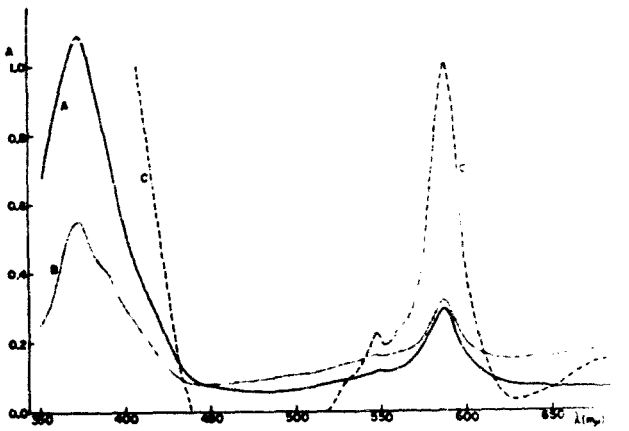


Fig. 11. Absorption spectra of anaerobic suspension of *R. rubrum* blue-green mutant cells. A, 298° K, optical path length 3 mm; B, 77° K, optical path length 1 mm; C, 77° K, optical path length 3 mm. 2.4 mg dry weight/ml in all cases.

The illuminated minus dark difference spectrum for these cells is illustrated by Fig. 12. Firstly, it is apparent that the region between 450 and 530 mμ is no longer obscured by carotenoid changes. A cytochrome(s) having a Soret band at 427 mμ was oxidized on illumination and an α-band at 553 mμ was also affected. It is likely that this change corresponds to the oxidation of cytochromes of *c*- and *b*-types, as has been suggested for the wild type *R. rubrum* on the kinetics of anaerobic light-off reaction. The existence of these two heme components in the blue-green mutant of *R. rubrum* is clearly shown by low-temperature spectra in a succeeding paper[17].

The components affected in the Soret region of the blue-green mutant have very nearly the same maximum as in the wild type. If one assumes that the cytochrome complement of the mutant is not appreciably changed, then we can interpret the identity of the major part of the change at 553 mμ in the wild type *R. rubrum* with that at 427 mμ. It would appear that the shape of the α-band observed in the studies of the wild type by CHANCE AND SMITH[5] is a little affected by carotenoids (spirilloxanthin and other components), but the β-band is largely affected by carotenoids as indeed was pointed out by those authors.

*Chromatium strain D*

The illumination-induced changes of the spectrum of this microorganism have been studied in detail by OLSON AND CHANCE[3-5]. It has been found, however, in washed *Chromatium* that illumination of the aerobic cells caused an increased absorption at 432 mμ (Fig. 13), which is similar in nature to that reported for washed *R. rubrum*[12], or PMA-treated aerobic cells of *R. rubrum* or *R. spheroides*[2,13]. This might be interpreted as the reduction of a heme protein by illumination when all the heme components are in the oxidized state.

When the washed aerobic cells of *Chromatium* were cooled down to 77° K with continuous illumination, the appearance of a band at 432 mμ by illumination was

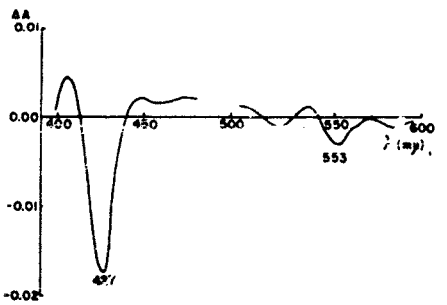


Fig. 12. Changes in absorption spectrum by illumination of blue-green mutant of *R. rubrum* (anaerobic suspension of intact cells). Optical path length, 10 mm; 2.4 mg dry weight/ml; temperature, 297° K.

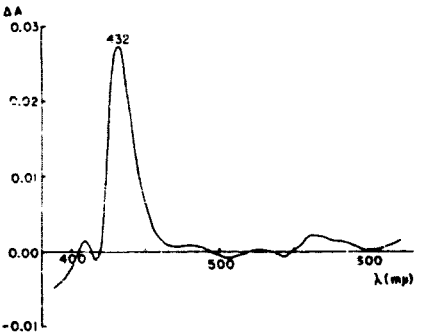


Fig. 13. Illuminated minus dark difference spectrum of washed aerobic *Chromatium* cells (washed four times and resuspended in 0.05 *M* phosphate buffer (pH 7.0)). Optical path length, 10 mm; temperature, 296° K.

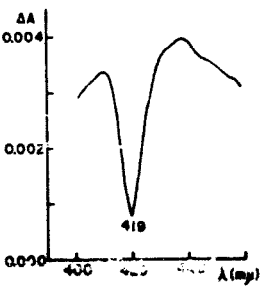


Fig. 14. Absorption spectrum change in washed *Chromatium* cells by illumination (illuminated continuously from room temperature through cooling to 77° K, measured at 77° K, optical path length 3 mm). Illuminated minus dark difference spectrum.

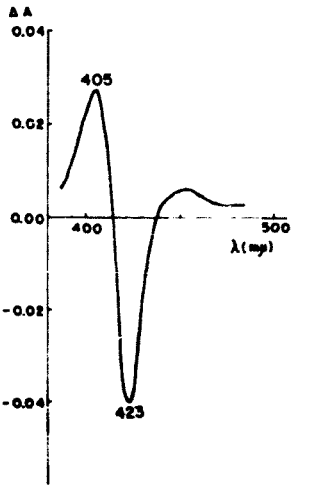


Fig. 15. Illuminated minus dark difference spectrum of washed anaerobic *Chromatium* cells (washed four times and resuspended in 0.05 *M* phosphate buffer (pH 7.0)). Temperature, 296° K; optical path length, 10 mm.

replaced by a decrease in absorption at 419 mμ (Fig. 14). This striking effect can be explained by the temperature-insensitivity of light-induced cytochrome *c* oxidation[10] and a larger temperature coefficient in the cytochrome-reducing system.

If slowly respiring washed *Chromatium* cells were allowed to become anaerobic, the absorbancy change reverted to one similar to that observed by OLSON AND CHANCE[3-5] as the "primary reaction". The record of this light response under these conditions is indicated in Fig. 15. It is seen that a cytochrome with a Soret band at 423 mμ was oxidized by illumination. The reversal of the direction of the light-induced absorption spectrum changes when the washed cells became anaerobic from aerobic state is shown in Fig. 16.

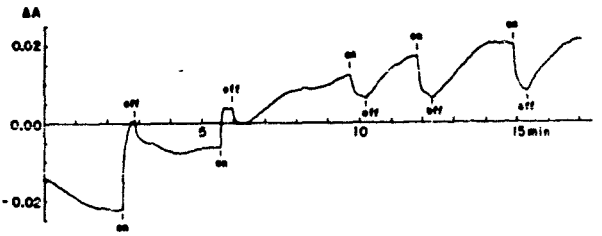


Fig. 16. Kinetics of absorption spectrum change by illumination in washed *Chromatium* cells (washed four times and resuspended in 0.05 *M* phosphate buffer (pH 7.0)), at 433 mμ. Aerobic suspension became anaerobic during measurement. Optical path length, 10 mm; temperature, 296° K.

*R. rubrum chromatophores*

Studies of light responses of cytochromes in the chromatophores are of considerable importance since the whole cell may show light responses not only of its chromatophores but also of its respiratory enzymes. This may occur in cells in which no oxygen is evolved in photosynthesis, by direct electron transfer or by the formation of high-energy intermediates which might initiate reversed electron transfer[29,30]. It has, however, been difficult to observe clear cytochrome responses in isolated *R. rubrum* chromatophores, although some progress has already been made[9,13].

Upon illumination, the chromatophores isolated from *R. rubrum* showed in both the aerobic and anaerobic states decreased absorption at 387 and 604 mμ which can be attributed to bacteriochlorophyll and increased absorption at 434 mμ (Fig. 17). The 434-mμ change was a broad band which had little resemblance to the band observed in the PMA-treated cells as reported here (see Figs. 3 and 10) or to the absorbancy changes observed in the starved cells[13] (*cf.* Fig. 13). In addition, carotenoids were still present and responded to illumination as indicated by the absorbancy changes in the region of 450–550 mμ. It was noticed that the 434-mμ band of Fig. 17 did not shift its peak at low temperature, and sharpening of the band at low temperature was not significant. These characteristics have not been observed in heme proteins[17,34,37,38]. The light-induced appearance of the 434-mμ band in *R. rubrum* chromatophores was not inhibited by potassium cyanide ($1 \cdot 10^{-3}$ *M*), PMA ($1 \cdot 10^{-4}$ *M*) or heptylhydroxyquinoline-*N*-oxide ($5 \cdot 10^{-5}$ *M*) and was observed in both aerobic and anaerobic chromatophore preparations.

The difference spectrum of the isolated chromatophores of *R. rubrum*, reduced minus aerobic, is illustrated in Fig. 18. Here is shown a large peak at 426 mμ which is

due to cytochromes[17]. While there is a suggestion of a cytochrome peak at 553 mμ, it appears that carotenoid pigments make this identification dubious.

As opposed to the kinetics of the light responses of the intact cells, rapid light-on and-off responses were obtained with the chromatophores and there was no evidence of a biphasic response. No difference of the response was obtained between the kinetics at 387 mμ, 434 mμ and 604 mμ (Fig. 19).

*Chromatium chromatophores*

The chromatophores prepared from *Chromatium* showed a light-induced response shown in Fig. 20 in which the oxidation of a cytochrome of *c*-type is indicated by a trough at 422 mμ with a corresponding α-band at 552 mμ. The absence of interference from carotenoids was characteristic of this microorganism. When the chromatophores were chemically reduced with dithionite, the reduced minus aerobic difference spectrum gave a typical cytochrome-type curve (Fig. 21). A Soret band at 425 mμ and an α-band at 553 mμ were observed. In comparison of this difference spectrum with the light-induced response (Fig. 20), the shift of the Soret band from 422 to

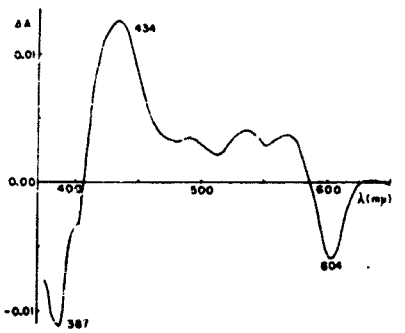


Fig. 17. Illuminated minus dark difference spectrum of isolated chromatophores from *R. rubrum*. Optical path length, 10 mm; temperature, 296° K.

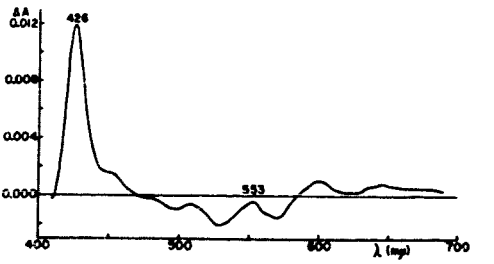


Fig. 18. Reduced minus aerobic difference spectrum of isolated chromatophores of *R. rubrum*. Optical path length, 10 mm; temperature, 296° K.

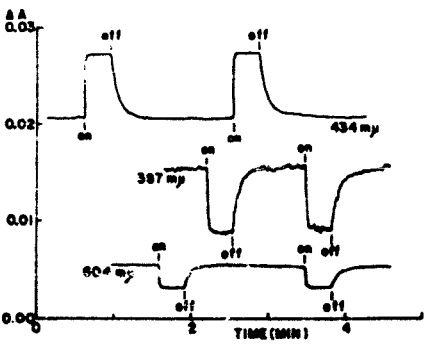


Fig. 19. Kinetics of light-induced absorption change of *R. rubrum* chromatophores. Absorption change by infrared illumination recorded at three fixed wavelengths: 387 mμ, 434 mμ and 604 mμ. Optical path length, 10 mm; temperature, 296° K.

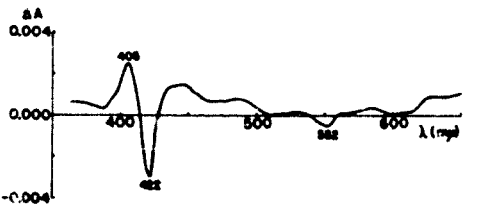


Fig. 20. Absorption spectrum change induced by illumination in isolated *Chromatium* chromatophores (illuminated minus dark difference spectrum). Optical path length, 10 mm; anaerobic; temperature, 297° K.

425 mμ could be due to the relatively small contribution of a cytochrome of *b* type in the light-induced oxidation in the isolated chromatophores of *Chromatium* (as in anaerobic starved *Chromatium* cells, Fig. 15).

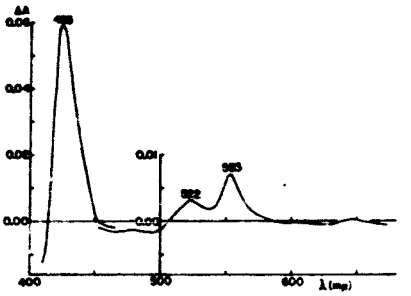


Fig. 21. Reduced (dithionite) minus aerobic difference spectrum of *Chromatium* chromatophores. Optical path length, 10 mm; temperature, 297° K.

## DISCUSSION

There are several ways of studying the mechanisms of electron transfer and light-induced absorption spectrum changes in photosynthetic bacteria. Three kinds of techniques have been adopted: (a) observation of difference spectra of suspensions of bacteria in two different steady states, in which conditions such as illumination, oxygen tension, substrates and inhibitors etc., are controlled, (b) analysis of the kinetics of changes induced by illumination, oxygen, etc., (c) isolation and reconstruction technique: cofactors, enzymes and particles taking part in photosynthetic and respiratory processes are isolated and studied in reconstructed systems. In this study, evidence obtained mainly by methods (a) and (b) have been presented, though results obtained by the third method are used to discuss the mechanism of electron transfer.

It would be convenient to present the evidence for the existence of each of the heme protein components in purple bacteria in a condensed statement. The presence of a *c*-type cytochrome ($c_2$) was shown by (a) isolation and purification[31-33], (b) light-induced absorption change in anaerobic cells[4,7,10], (c) PMA-induced absorption change in aerobic cells[2,17], (d) absorption spectrum change induced by heptylhydroxyquinoline-*N*-oxide or antimycin A in anaerobic cells[25]. The presence of a *b*-type cytochrome was demonstrated by (a) isolation and purification[34], (b) absorption spectrum change by oxygen introduction[17], (c) light-induced absorption spectrum change in PMA-treated aerobic cells (*R. rubrum* and *R. spheroides*) or in washed aerobic cells (*Chromatium*), the fact that carbon monoxide did not interfere with this change indicates the presence of a *b*-type cytochrome besides a CO-binding pigment. The presence of a CO-binding pigment, RHP/cytochrome *o* was demonstrated by the following data: (a) isolation and purification[31-33,35], (b) absorption spectrum change caused by carbon monoxide[2,17], (c) photochemical action spectrum of CO-inhibited respiration which is of cytochrome *o* type and similar to the spectrum of RHP–CO complex[17,36].

Controversy on the appearance of a band(s) around 430 mμ by illumination in the chromatophores or the aerobic cells has been somewhat difficult to resolve (see INTRODUCTION). In our study, there seem to be present two different phenomena: one is the appearance of a band with its peak at 429 to 432 mμ (at room temperature;

the peak positions show little variations in different species) in several photosynthetic bacteria on illumination when the cells are aerobic (washed *Chromatium* cells) or treated with PMA (*R. rubrum* and *R. sphcroides*); the other is observed in the isolated *R. rubrum* chromatophores upon illumination of both anaerobic and aerobic suspensions. In the latter case, a broad band appears at 434 mμ and large troughs, perhaps corresponding to absorption spectrum change of bacteriochlorophyll, appear at 387 and 604 mμ. Similar changes were observed in green plants by WITT *et al.*[37-39] and KOK[40-42].

In the *R. rubrum* chromatophores, the 434-mμ band was very broad and the band location did not change by cooling to 77° K. On the other hand, the changes which aerobic cells or PMA-treated cells underwent had sharper maxima at low temperature. In the latter case the positions of maxima were shifted towards shorter wavelengths, as usually observed in cytochromes[24,27,38]. Kinetics of the changes were also different in these two examples. This evidence suggests two distinct mechanisms for the appearance of a band near 430 mμ, one for *R. rubrum* chromatophores and another for other cases. Reduction of a *b*-type cytochrome by illumination in the aerobic or PMA-treated cells explains the latter cases.

We do not have enough evidence to determine the chemical nature of the 434-mμ band in the illuminated *R. rubrum* chromatophores. OLSON AND KOK[43] showed that the appearance of a 436-mμ band by illumination in aerobic *Chromatium* was not synchronous with changes of infrared absorption of bacteriochlorophyll, and they refuted the hypothesis presented by DUYSENS *et al.*[44] that the changes at 436 mμ and in the near infrared are caused by one reaction, specifically oxidation of bacteriochlorophyll. In OLSON AND KOK's observations there were no significant absorption changes between 600 mμ and 740 mμ, so their results do not correspond to the absorption changes in *R. rubrum* chromatophores but probably correspond to the change observed in aerobic suspensions of several photosynthetic bacteria. At present, we are inclined to attribute the decreased absorption at 387 and 604 mμ and the increased absorption at 434 mμ by illumination in *R. rubrum* chromatophores to a single reaction, *viz.* change of bacteriochlorophyll spectrum, which is unlikely to participate in the electron transfer in intact cells. ARNOLD AND CLAYTON[45] indicated that this change is electronic and suggested that the shift is diminished in the intact cells to the extent that enzymes can remove the primary excited electrons and positive holes. It is still to be decided whether this electronic change corresponds to an accumulation of primary excited electrons and holes which can be transferred to the electron transport system or whether this is a secondary phenomenon induced by the charge separation. In intact cells the first substance to be oxidized by accepting a positive hole (or giving an electron) has been found to be cytochrome *c* by the present authors[10,11].

The temperature-insensitivity of cytochrome *c* oxidation in *Chromatium* cells by infrared illumination suggests the process for direct positive hole (or electron) transfer between bacteriochlorophyll and cytochrome molecules without intermolecular collisions[10,11]. The slight temperature-dependence of the light-induced cytochrome oxidation in the *Chromatium* chromatophores might be a result of poorer association between bacteriochlorophyll and cytochrome molecules, though the dependence is small and we could observe the light-induced change at 205° K. Temperature-sensitive light-induced reactions were observed in *R. rubrum* and

*R. spheroides*. The sensitivity is probably due to the decrease of the reduced form of heme proteins in the cells cooled to lower temperatures. The difference of the temperature coefficients between reductase and oxidase systems might affect the steady-state oxidation level of cytochromes upon cooling.

It is unlikely that carotenoids are situated in the main path of electron transfer in the photosynthetic and respiratory systems because other electron carriers function normally in carotenoidless mutants of *R. spheroides* and *R. rubrum*. There is no indication of participation of carotenoids in *Chromatium* either. But the changes in the carotenoid absorption spectrum induced by light or oxygenation suggest to us that the change in the absorption spectrum of carotenoids is caused by the flow of electrons between the carotenoid molecule and other electron carriers. The change in the carotenoid steady state caused by addition of antimycin A or heptylhydroxyquinoline-*N*-oxide[26] also suggests this possibility. A similar change in the steady state of carotenoids was also observed in *R. spheroides* when the anaerobic cells were subjected to cooling[46]. These data can be interpreted as a demonstration of different temperature coefficients or different sensitivities to inhibitors in electron-donating and-accepting systems for carotenoids, rather than the result of different sensitivities of carotenoids directly to oxygen or light in different conditions. The apparent absorption band shift of 16–17 mμ towards longer wavelengths in the difference spectrum (the real band shift might possibly be a few mμ smaller) can be attributed to an increase of the effective length of π-electron system in the conjugated polyene chain (addition of one C–C unit to the conjugated system) or an introduction of a bathochromatic group (addition of –OH, etc.).

The characteristics of heme protein components in photosynthetic bacteria are discussed in detail in the succeeding papers[17,26]. It is indicated that in the light-induced electron transport, the *c*-type cytochrome accepts positive holes from (or transfers electrons to) the excited bacteriochlorophyll molecules (primary photochemical process?). On the other hand, the *b*-type cytochrome is located nearer to the photochemical reducing site, and its reduction by illumination is observed in PMA-treated *R. rubrum* and *R. spheroides* or washed aerobic *Chromatium*. The presence of heptylhydroxyquinoline-*N*-oxide or antimycin A blocks the reaction between these two heme components in photosynthetic and respiratory electron transport and induces an interesting crossover phenomenon[26]. A CO-binding pigment, RHP/cytochrome *o* has been shown to be active as oxidase in dark-grown *R. rubrum*[17,26]. The fact that carbon monoxide did not inhibit the photochemical reduction of the *b*-type cytochrome suggests that RHP/cytochrome *o* is not located between the cytochrome *b* and the photochemical reducing site.

The presence of ubiquinone/coenzyme Q in photosynthetic bacteria is reported by LESTER AND CRANE[47]. In both the wild and the blue-green mutant of *R. rubrum*, we[11] obtained data which suggest the aerobic-anaerobic transition of the substance. HORIO AND KAMEN[48] reported the pyridine nucleotide–hemoprotein reductase and photophosphorylation-activating protein. Both (same protein?) seem to contain flavin. BALTSCHEFFSKY's findings[49] that FAD restored photophosphorylation activity of quinacrine-inhibited *R. rubrum* chromatophores also suggests participation of flavin in photosynthetic bacteria.

FRENKEL[50,51], DUYSENS *et al.*[52–54], VERNON[55–57] and OLSON *et al.*[58–60] reported the light-induced reduction of pyridine nucleotide in purple bacteria and chromato-

phores. It is likely that pyridine nucleotides and flavoproteins are located near the photochemical reducing site in the photosynthetic redox chain (directly or indirectly). The light-induced reduction of the *b*-component of cytochrome under certain conditions might occur through these electron carriers in photosynthetic bacteria.

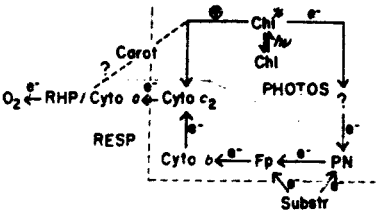


Fig. 22. Scheme of electron-transferring system in *R. spheroides* and *R. rubrum*. *Chromatium* and blue-green mutants of *R. spheroides* and *R. rubrum* have essentially the same system with the exception of evidence of carotenoid participation.

Considering these results we postulate a scheme for the electron transferring system in purple bacteria (Fig. 22). The wild strains of *R. rubrum* and *R. spheroides* have essentially the same system, whereas *Chromatium* and the blue-green mutants of *R. spheroides* and *R. rubrum* seem to have the same system except that there is no evidence of carotenoid participation in these strains. The role of RHP/cytochrome *o* in the strict photoanaerobe *Chromatium* is still the subject of further investigation.

## REFERENCES


[1] L. N. M. DUYSENS, *Nature*, 173 (1954) 692.
[2] B. CHANCE AND L. SMITH, *Nature*, 175 (1955) 803.
[3] J. M. OLSON, *Research in Photosynthesis*, Interscience, New York, 1957, p. 174.
[4] J. M. OLSON AND B. CHANCE, *Arch. Biochem. Biophys.*, 88 (1960) 26.
[5] J. M. OLSON AND B. CHANCE, *Arch. Biochem. Biophys.*, 88 (1960) 40.
[6] B. CHANCE, *Brookhaven Symposia in Biology No. 11*, 1959, p. 74.
[7] I. SMITH AND J. RAMÍREZ, *Arch. Biochem. Biophys.*, 79 (1959) 233.
[8] L. SMITH AND J. RAMÍREZ, *J. Biol. Chem.*, 235 (1960) 219.
[9] L. SMITH AND M. BALTSCHEFFSKY, *J. Biol. Chem.*, 234 (1959) 1575.
[10] B. CHANCE AND M. NISHIMURA, *Proc. Natl. Acad. Sci. U.S.*, 46 (1960) 19.
[11] B. CHANCE AND M. NISHIMURA, *Proc. 5th Intern. Congr. Biochem., Moscow, 1961*, in the press.
[12] L. N. M. DUYSENS, *Research in Photosynthesis*, Interscience, New York, 1957, p. 164.
[13] B. CHANCE, M. BALTSCHEFFSKY AND L. SMITH, *Research in Photosynthesis*, Interscience, New York, 1957, p. 192.
[14] L. SMITH, M. BALTSCHEFFSKY AND J. M. OLSON, *J. Biol. Chem.*, 235 (1960) 213.
[15] H. GEST, M. D. KAMEN AND H. M. BREGOFF, *J. Biol. Chem.*, 182 (1950) 153.
[16] G. COHEN-BAZIRE, W. R. SISTROM AND R. Y. STANIER, *J. Cellular Comp. Physiol.*, 49 (1957) 25.
[17] M. NISHIMURA AND B. CHANCE, *Studies on Microalgae and Photosynthetic Bacteria (Plant & Cell Physiol. Special Issue)*, in the press.
[18] J. W. NEWTON AND M. D. KAMEN, *Biochim. Biophys. Acta*, 21 (1959) 71.
[19] M. NISHIMURA, *Biochim. Biophys. Acta*, 57 (1962) 88.
[20] C.-C. YANG AND V. LEGALLAIS, *Rev. Sci. Instr.*, 25 (1954) 801.
[21] C.-C. YANG, *Rev. Sci. Instr.*, 25 (1954) 807.
[22] B. CHANCE, *Rev. Sci. Instr.*, 22 (1951) 634.
[23] B. CHANCE, *Science*, 120 (1954) 767.
[24] R. W. ESTABROOK, *J. Biol. Chem.*, 223 (1956) 781.
[25] B. HAGIHARA, *Biochim. Biophys. Acta*, 46 (1961) 134.
[26] M. NISHIMURA, *Biochim. Biophys. Acta*, 66 (1963) 17.
[27] R. W. ESTABROOK AND B. MACKLER, *J. Biol. Chem.*, 224 (1957) 637.
[28] R. W. ESTABROOK AND B. SACKTOR, *Arch. Biochem. Biophys.*, 76 (1958) 532.
[29] B. CHANCE AND G. HOLLUNGER, *Nature*, 185 (1960) 666.

[30] B. CHANCE AND G. HOLLUNGER, *J. Biol. Chem.*, 236 (1961) 1534; 1544; 1555; 1562, 1569; 1577.
[31] L. P. VERNON AND M. D. KAMEN, *J. Biol. Chem.*, 211 (1954) 643.
[32] M. D. KAMEN AND L. P. VERNON, *Biochim. Biophys. Acta*, 17 (1955) 10.
[33] R. B. BARTSCH AND M. D. KAMEN, *J. Biol. Chem.*, 235 (1960) 825.
[34] J. A. ORLANDO AND T. HORIO, *Biochim. Biophys. Acta*, 50 (1961) 367.
[35] R. G. BARTSCH AND M. D. KAMEN, *J. Biol. Chem.*, 230 (1958) 41.
[36] C. P. S. TAYLOR AND T. HORIO, personal communication.
[37] H. T. WITT, R. MORAW, A. MÜLLER, B. RUMBERG AND G. ZIEGLER, *Z. Elektrochem.*, 64 (1960) 181.
[38] H. T. WITT, R. MORAW, A. MÜLLER, B. RUMBERG AND G. ZIEGLER, *Z. phys. Chem.*, 23 (1960) 133.
[39] H. T. WITT, A. MÜLLER AND B. RUMBERG, *Nature*, 191 (1961) 194; 192 (1961) 967.
[40] B. KOK, *Biochim. Biophys. Acta*, 22 (1956) 399.
[41] B. KOK, *Acta Botan. Neerl.*, 6 (1957) 316.
[42] B. KOK, *Plant Physiol.*, 34 (1959) 184.
[43] J. M. OLSON AND B. KOK, *Biochim. Biophys. Acta*, 32 (1959) 278.
[44] L. N. M. DUYSENS, W. J. HUISKAMP, J. J. VOS AND J. M. VAN DER HART, *Biochim. Biophys. Acta*, 19 (1956) 188.
[45] W. ARNOLD AND R. K. CLAYTON, *Proc. Natl. Acad. Sci. U.S.*, 46 (1960) 769.
[46] B. CHANCE AND M. NISHIMURA, unpublished data.
[47] R. L. LESTER AND F. L. CRANE, *J. Biol. Chem.*, 234 (1959) 2169.
[48] T. HORIO AND M. D. KAMEN, *Biochim. Biophys. Acta*, 43 (1960) 382.
[49] H. BALTSCHEFFSKY, *Biochim. Biophys. Acta*, 40 (1960) 1.
[50] A. W. FRENKEL, *Brookhaven Symposia in Biology No. 11*, 1958, p. 276.
[51] A. W. FRENKEL AND D. D. HICKMAN, *J. Biophys. Biochem. Cytol.*, 6 (1959) 285.
[52] L. N. M. DUYSENS AND G. SWEEP, *Biochim. Biophys. Acta*, 25 (1957) 13.
[53] J. M. OLSON, L. N. M. DUYSENS AND G. H. M. KRONENBERG, *Biochim. Biophys. Acta*, 36 (1959) 125.
[54] L. N. M. DUYSENS AND J. AMESZ, *Plant Physiol.*, 34 (1959) 210.
[55] L. P. VERNON, *J. Am. Chem. Soc.*, 80 (1958) 246.
[56] L. P. VERNON, *J. Biol. Chem.*, 233 (1958) 212.
[57] L. P. VERNON AND O. K. ASH, *J. Biol. Chem.*, 234 (1959) 1878.
[58] J. M. OLSON, *Brookhaven Symposia in Biology No. 11*, 1959, p. 316.
[59] J. M. OLSON AND J. AMESZ, *Biochim. Biophys. Acta*, 37 (1960) 14.
[60] B. CHANCE AND J. M. OLSON, *Arch. Biochem. Biophys.*, 88 (1960) 54.