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SOME PROPERTIES OF PIGMENT-PROTEIN COMPLEXES IN PURPLE BACTERIA

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1. INTRODUCTION

In connection with attempts at separation of the different complexes supposed to be present in the purple bacteria and manifest by different absorption maxima in the near infrared, we also endeavoured to further characterize the different pigment-protein complexes by studying effects on harvested cells and during growth of cultures. The effects of culture conditions upon the near infrared absorption spectra are the subject of this paper, as a continuation of earlier work (1, 2).

We found that in our *Chromatium*, strain D the near infrared absorption spectrum, which in normal cells is of a multipeak type, has retained its multipeak character after growth on diphenylamine (DPA), which causes carotenoid deficiency (3).

The occurrence of the normal spectrum also in carotenoid-deficient cells (the same holds for the pigment-protein complexes present in colloidal extracts of both types, prepared by grinding or ultrasonics) is at variance with the observations of BERGERON and FULLER (4). These authors found a strong reduction of absorption in the long wavelength region, accompanying the great reduction of the absorption in the 450–550 mμ region of the spectrum, owing to carotenoid deficiency. Their hypothesis is that the multipeak type of the near infrared absorption spectrum is not due to bacteriochlorophyll complexes alone, but that it is produced by interaction between bacteriochlorophyll and carotenoids. This interaction is supposed to change when the strongly conjugated coloured carotenoids are replaced by less conjugated precursors by the action of DPA. This should change the near infrared absorption spectrum to a more pronounced one peak type, supposed to originate from bacteriochlorophyll alone. A simular

suggestion was made by STANIER et. al. (5) in the case of a carotenoid-less mutant of *Rhodopseudomonas spheroides*.

Another difference between normal and carotenoid-deficient *Chromatium*, is the greater sensitivity to photo-oxidation of the latter (6, 7, 8, 9), as found in *Rhodospirillum rubrum* and *Rhodopseudomonas spheroides* (5, 10, 11), and also the greater sensitivity to extraction by organic solvents (8).

As stated before (3), the coincidence of disappearance of carotenoids and increased sensitivity to photo-oxidation, need not necessarily be explained by direct protection of bacteriochlorophyll by carotenoids against photo-oxidation. The effect of red plus near infrared light in photo-oxidation in normal and carotenoid-deficient *Chromatium* cells and colloidal extracts, is the same as that for white light (also absorbed by carotenoids, 8) so that direct photochemical protection by carotenoids appears less probable. Combined with inhibition of the synthesis of coloured carotenoids, structural changes in the chromatophores may occur in the presence of DPA. The fact that bacteriochlorophyll is more easily extracted from carotenoid-deficient *Chromatium* cells by several organic solvents, compared to extraction of normal cells, seems to support the assumption of structural differences between both types (8).

The question which had occupied us for a long time, is the mentioned discrepancy between the shapes of the absorption spectra of carotenoid-deficient *Chromatium*, strain D as observed by FULLER and by ourselves. At first, an explanation seemed found in the fact that our *Chromatium* culture appeared to contain a *Rhodopseudomonas* type of bacteria, as kindly mentioned to us by Professor FULLER in a personal information. However, a further study of a number of different cultures made clear that the presence of an admixture was not the final explanation of our results, as will be demonstrated in this paper. This further study included various cultures listed *Chromatium*, strain D, partly of different origin, e.g., the *Chromatium* strain reisolated from our original culture, and a culture placed at our disposal by Professor FULLER, but also a *Rhodopseudomonas spheroides* culture and a *Rhodopseudomonas*-like culture obtained by purification of the admixture isolated from our original culture.

BRIL (9) neither found differences in absorption in the long wavelength region, except a decreased 890 mμ shoulder, after growth of *Chromatium* in the presence of 12 mgm DPA/l, thus giving support for our view (3) that the general type of the near infrared absorption spectrum is not affected by DPA.

2. MATERIAL AND METHODS

Chromatium, strain D cultures were grown in 100 ml sterile, completely filled, glass-stoppered culture bottles in the incandescent light cabinet either in malate-thiosulfate or in carbonate-thiosulfate medium both in the absence or presence of DPA in concentrations up to 30 mgm per liter, under normal and low light conditions. In some experiments a red transparant paper was placed around the bottles to suppress light of shorter wavelengths.

The malate-thiosulfate medium consisted of 1.0% NaCl, 1.0% rock-salt,

0.1% $(NH_4)_2SO_4$, 0.05% K_2HPO_4, 0.02% $MgSO_4$, 0.015% Na_2S, 0.15% $Na_2S_2O_3$ and 0.23% L (–) sodium-malate, with the addition of NaOH or H_3PO_4, as far as necessary, to adjust pH at 7.6. In this medium an abundant growth generally occurred. The carbonate-thiosulfate medium consisted of the same components except the replacement of malate by 0.5% $NaHCO_3$. In this medium growth was considerably reduced as compared to that in the malate medium.

Chromatium cultures were obtained from Professor J.B. THOMAS, Utrecht, and Professor L.N.M. DUYSENS, Leiden. Like our own, these cultures descend from a culture, supplied in 1935 by Dr.P.A. ROELOFSEN, and maintained at Utrecht until 1947 by Dr.E.C. WASSINK. Another culture was obtained from Professor R.C. FULLER, which originally came from Professor C.B. VAN NIEL. Moreover, a strain, called Wageningen culture, was reisolated from our original (mixed) culture. The actual reisolation was preceded by cultivation during several generations in a medium, containing DPA up to 30 mgm per liter, in which *Chromatium* obviously developed better than the admixture. After several transfers and shake cultures a strain was obtained which developed the stair-case type of absorption spectrum during growth at low light intensity, in the same way as the other cultures tested, thus reproducing earlier results (1, 2).

The normal light intensity in the light cabinet, supplied with Tungsten lamps, varied from about 2500 to 10.000 ergs/cm² sec. depending on the spot. Decrease of light intensity to a few per cent of the initial value, was provided as before (2) by paper covers on the bottles, causing non-selective weakening.

The admixture was isolated via shake cultures by application of a medium containing 1.0% NaCl, 1.0% rock-salt and 1.0% bacto-peptone, pH adjusted to 7.4 with NaOH or HCl. *Rhodopseudomonas spheroides* obtained from Professor J.B. THOMAS, Utrecht, and the isolated admixture were both grown either on this peptone-NaCl medium or on a medium containing 0.5% yeast extract, 0.5% $MgSO_4$, 0.3% sodium-malate in 0.02 M phosphate buffer with addition of ammonia to adjust pH at 6.8. Growth of both cultures was possible on media containing DPA in concentrations up to 15 mgm per liter.

The L-malic acid used was from Nutritional Biochemicals Corporation, bacto-peptone and yeast-extract from Difco Laboratories Incorporated, and diphenylamine (pro anal.) from Merck Aktiengesellschaft.

Absorption measurements were made with a handoperated Beckman DU spectrophotometer. A sheet of filter paper according to SHIBATA (13) was inserted into the light beam to minimize the effect of scattering of the cells on the spectrum, which in several cases is extensive, especially when sulfur is present in the cells. The absorption spectra in one figure always are of cultures from the same inoculum.

3. RESULTS

a. Comparison of different cultures, listed Chromatium, *strain D*

a-1. Growth under normal light intensities. Cultures of the Leiden, Utrecht and Wageningen strains often develop very similar absorption spectra.

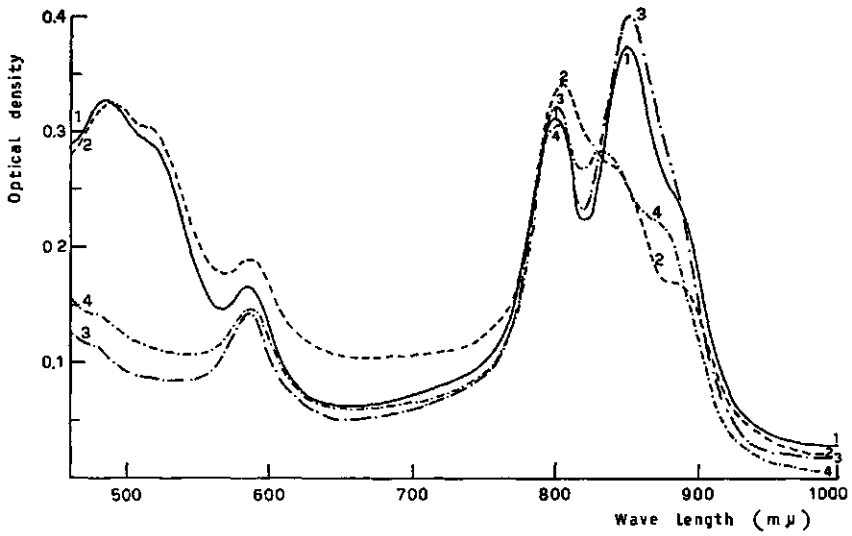

FIG. 1. Absorption spectra of *Chromatium*, strain D, Wageningen culture, on L-malate at normal (1) and low light intensities (2); and in the presence of 20 mgm DPA per liter at normal (3) and low light intensities (4).

As a rule the absorption spectrum of the FULLER culture had a more pronounced 890 mμ shoulder, especially when carbonate was supplied instead of malate; this confirms data of FULLER (12). As reported earlier (3), in the presence of DPA the near infrared absorption spectrum of the Wageningen culture remains of the two-peak type. It should be especially emphasized that the same holds for the newly isolated Wageningen *Chromatium* strain (fig. 1) and also for the Leiden, Utrecht and FULLER cultures (fig. 2). Using DPA concentrations from 0 to 30 mgm DPA per liter the near infrared spectrum mostly was the two-peak type, only with an appreciably decreased 890 mμ shoulder, as found before (3). We were not able to cultivate these strains at concentrations above 30 mgm per liter. Growth in the presence of DPA is strongly reduced, often a heavy inoculum is necessary to obtain any growth at all at the highest concentrations.

In some experiments, the 850 mμ absorption was somewhat decreased. It should be mentioned that this may also occur without DPA at normal light intensities (fig. 3). In most cases, the spectrum clearly retained the two-peak type, with decreased 890 mμ absorption; carotenoid deficiency was manifest by decreased absorption in the 450–550 mμ region. The 850 mμ peak still was higher than or equally high as the 800 mμ peak. In cases in which the 850 mμ peak incidentally was strongly reduced, it showed an apparent shift to somewhat shorter wavelengths. In some cases, the near infrared absorption spectrum resembled the stair-case type of spectrum of DPA-free cells, grown e.g., under low light conditions. (It has been mentioned already that conditions other than reduced light intensity, occasionally produce the stair-case spectrum.) In the latter case, however, the absorption in the carotenoid part of the spectrum is

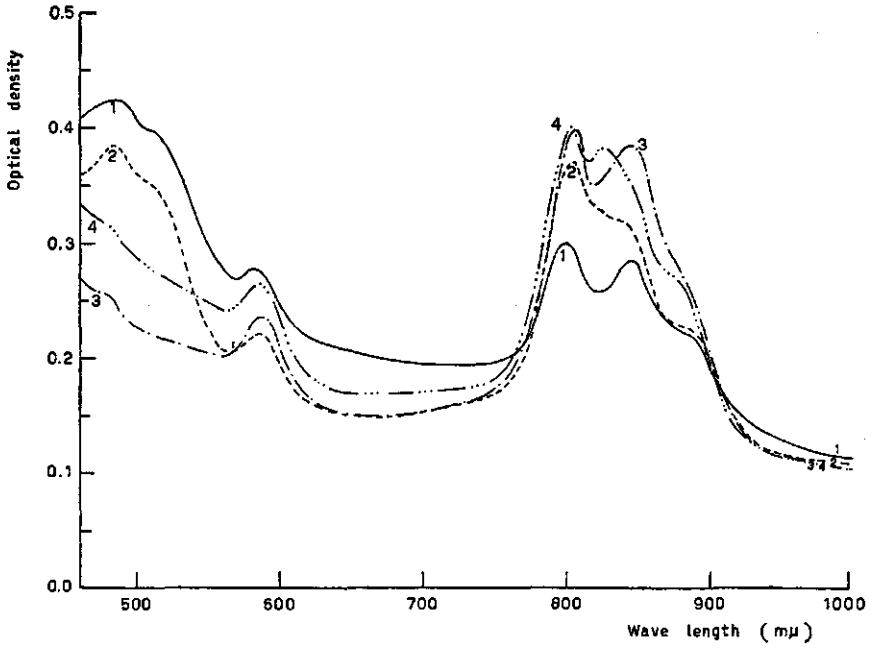

FIG. 2. Absorption spectra of *Chromatium*, strain D, FULLER culture, on L-malate at normal (1) and low light intensities (2); and in the presence of 20 mgm DPA per liter at normal (3) and low light intensities (4).

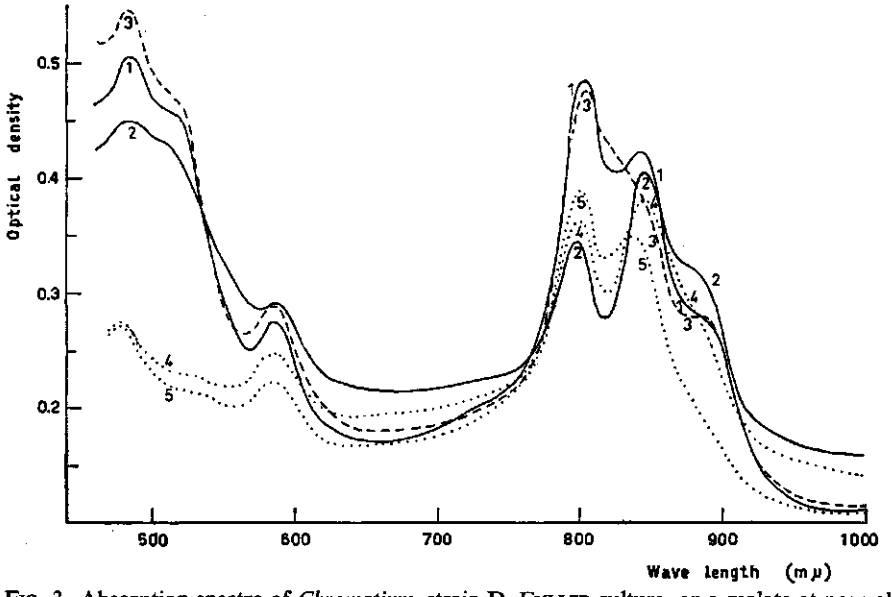

FIG. 3. Absorption spectra of *Chromatium*, strain D, FULLER culture, on L-malate at normal (1, 2) and low light intensity (3); and on L-malate at normal light intensities in the presence of 20 mgm DPA per liter (4, 5).

not appreciably reduced, and it seems likely that shifts as reported by BERGERON and FULLER, at least in part, are more closely related to conditions giving rise to a 'stair-case spectrum' than to those producing carotenoid deficiency.

a-2. Growth under reduced light intensity. The only definite condition causing reproducible differences in the absorption spectrum during growth, detected so far, is low light intensity. This causes a decrease of the 850 mμ peak to well below the 800 mμ one. The long wavelength shoulder at about 890 mμ becomes more prominent in connection with the decrease of the 850 mμ maximum. Aside of the variation in height of the 850 mμ peak, the spectra of cells, grown under normal and low light conditions also differ in the height of the 800 mμ maximum; often this peak in the stair-case spectrum is higher than in the normal one. All *Chromatium*, strain D cultures, used so far, viz., the Utrecht, Leiden, Wageningen and FULLER cultures, developed the stair-case type of spectrum when light intensity during growth was considerably reduced. When malate was replaced by carbonate, at low light intensity, all strains tended to show a spectrum intermediate between that of cells grown with malate at low light intensity, and that of normally grown cells (fig. 4).

Under low light conditions, the stair-case type of spectrum always appeared also in the presence of DPA. However, the decrease of the 850 mμ peak sometimes is less evident than in the real stair-case spectrum. The 890 mμ shoulder, usually decreasing in the presence of DPA, appeared more pronounced when DPA was applied at low light intensity. This may be due to the decrease of the 850 mμ peak, which usually is of normal height in the presence of DPA at high light intensity. The stair-case type of the spectrum in the presence of DPA at low light intensities, however, was somewhat less pronounced than as published by BERGERON and FULLER (4) and shown in figures received in a personal communication; these data were obtained at high DPA concentrations and without decrease of light intensity.

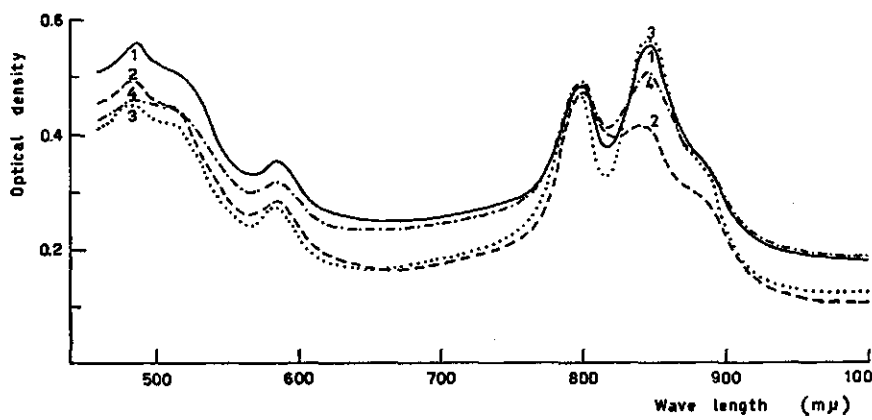

FIG. 4. Absorption spectra of *Chromatium*, strain D, Wageningen culture, on L-malate at normal (1) and low light intensities (2); on carbonate at normal (3) and low light intensities (4).

Low light conditions with carbonate and DPA gave rise to a less pronounced stair-case type of spectrum than observed in the presence of malate and the same concentration of DPA.

Concluding, we may say that in the presence of DPA a stair-case type of spectrum appears if other conditions favour this, thus e.g., low light intensities. This holds for both the Wageningen and the FULLER strain. The reason why low light conditions cause this stair-case type of spectrum, and why this tendency is less in the presence of carbonate is so far unexplained (an object of further investigation).

b. Properties of the 'admixture'

The absorption spectrum of the admixture cells showed a close resemblance with that of *Rhodopseudomonas spheroides.* Aeration of a culture of *Rhodopseudomonas spheroides* during several hours in the dark, turned its brownish colour into a more reddish hue, as is well known (2); the same was found with our isolation. The results of experiments with both strains under different culture conditions in the presence or absence of DPA and under different light conditions, were very much the same. The absorption spectrum of the admixture grown at normal light intensity showed a very high 850 mμ peak as compared to the 795 mμ one, a very deep valley between the two peaks while the 890 mμ shoulder often was very small (fig. 5). After growth under reduced light intensity, the spectrum was not changed into a stair-case spectrum as in *Chromatium*; the high 850 mμ peak remained and the relation in height of the two main near infrared absorption maxima (E850/E795) is rather unchanged or sometimes even increased, thus opposite to the situation in *Chromatium* at low light intensities. In the presence of DPA (up to 15 mgm per liter; in higher concentrations no development was observed), the E850/E795 relation neither was decreased, even often increased, the largest effect occurring at the highest DPA

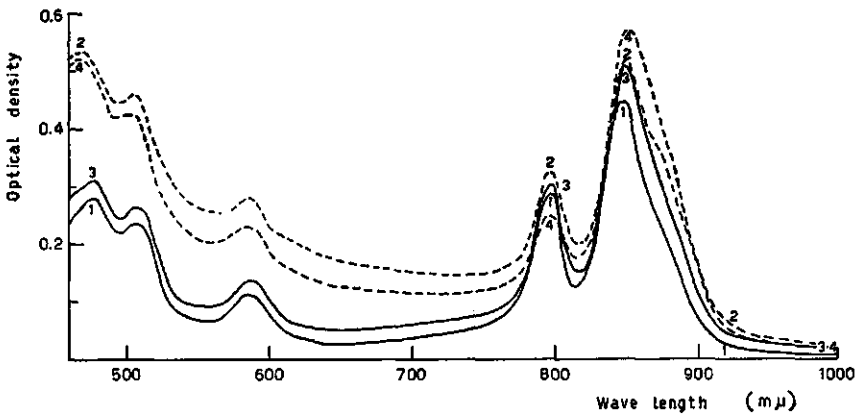

FIG. 5. Absorption spectra of the admixture in malate-yeast medium. At normal (1) and low light intensities (2); and with 15 mgm DPA per liter at normal (3) and low light intensities (4).

concentrations at which some growth still took place. Addition of DPA under low light conditions did not decrease the 850 mμ absorption peak, the increase of the E850/E795 relation observed in the presence of DPA, was often even more pronounced under low light conditions.

Carotenoid deficiency which should manifest itself by striking spectral changes in the 450–550 mμ part of the spectrum as compared to cells grown in the absence of DPA, was not observed. It should be remarked that neither in any of the *Chromatium*, strain D cultures, nor in the *Rhodopseudomonas* type, wavelenght shifts of the infrared peaks were observed in connection with carotenoid deficiency, except if at the same time a stair-case type spectrum arose which, however, had to be attributed to causes other than carotenoid deficiency.

4. Discussion

Several observations coincided in demonstrating that the *Chromatium* culture we used for obtaining preliminary results (3), deviated from that originally used (1, 2) by the presence of an admixture of a *Rhodopseudomonas* cell type. The 850 mμ peak in the *Rhodopseudomonas* at low light intensities, is at least as high or higher than that under normal culture conditions (fig. 5). This effect is opposite to the decrease, of the 850 mμ maximum observed in *Chromatium* (fig. 1, 2, 3, 4). Therefore, a mixture of both species may fail to produce a real stair-case spectrum at low light intensities. This explains why in previous experiments with our original culture at low light intensity the 850 mμ peak was about equally high as the 800 mμ one.

Wassink et. al. (2) made reasonably sure that the two types of absorption spectra in *Chromatium*, strain D cultures, viz. at low and at high light intensities are not due to two initially different cell types or species. One may assume that in each cell the nature of the pigment-protein complex depends on the environmental conditions during growth, while once built up, the complex is largely insensitive to changes in the external conditions (2). The observation of Fuller (12) of differentiation of the photochemical apparatus into apparently independent chromatophores, accompanying an increase in pigment and total lipid at low light intensities, may be considered to support the latter view.

With regard to the effect of the presence or absence of carotenoids on the near infrared absorption spectrum of *Chromatium*, our experiments with the Utrecht, Leiden, Fuller and the purified Wageningen culture of *Chromatium*, strain D, all had the same results as described before (3). In general, the near infrared absorption spectrum of carotenoid-deficient *Chromatium* is of the two-peak type (fig. 1, 2). Sometimes an unexpected decrease of the 850 mμ peak is observed, which may also occur, however, in normal cells, even at normal light intensities, without any appreciable change in carotenoid absorption (fig. 3). It thus appears unlikely that the absence of carotenoids produces considerable changes in the near infrared part of the spectrum.

It might be asked whether a mixture of *Chromatium* and *Rhodopseudomonas*, as present in the culture used in our first experiments in the presence of DPA

may develop about the same spectrum as normal cells, the carotenoid-deficient *Chromatium* cells showing reduced absorption in the 890–850 mμ region whilst in *Rhodopseudomonas* cells the 850 mμ absorption peak still is high or even increased. The resulting spectrum of the mixture then may still show the two-peak-type. However, our observations with our purified strain and those from other origins seem to speak strongly against this possibility, so that contribution of carotenoids to the near infrared spectrum of *Chromatium* in the way suggested by BERGERON and FULLER (4) and STANIER (5) seems unlikely.

Our results with *Chromatium* grown in the presence of DPA at low and normal light intensities, indicate that the strong decrease of absorption in the 850 mμ region as observed by BERGERON and FULLER (4) are due to conditions giving rise to a stair-case type of absorption spectrum, rather than to the presence of DPA. Notwithstanding the possibility that thick growth in the presence of DPA in a large bottle might equal the effect of low light intensity and produce decreased absorption in the 850 mμ range, this, does not offer a probable explanation since without DPA a stair-case type of spectrum generally did not develop in FULLER's experiments (4).

Thus, in the presence of DPA appreciable changes in the bacteriochlorophyll spectrum are not observed except incidental ones, also possible under normal conditions. However, there seems to be a structural difference compared with normal cells, which renders the green cells more sensitive to photo-oxidation and extraction (8, 9).

It is not clear yet whether the differences observed in carbonate media, viz. the more pronounced 890 mμ shoulder and the absence of a real stair-case spectrum under low light intensity, are due to structural changes.

In the presence of DPA the *Rhodopseudomonas* isolation might show an increase of absorption in the 875 mμ region since a blue-green mutant of *Rhodopseudomonas spheroides* has its highest maximum at this wavelength (5). However, small concentrations of DPA at which some growth of *Rhodopseudomonas* still exists, probably do not produce carotenoid deficiency in this species. This is indicated by the fact that no striking changes in the carotenoid part of the absorption spectrum are observed, as there are in *Chromatium*. Carotenoid deficiency and striking changes in the near infrared spectrum as found in the blue-green mutant, might occur at higher DPA concentrations; however, the very scanty growth under these conditions up to now interferes with this sort of observation.

5. SUMMARY

This investigation deals with further properties of pigment-protein complexes in *Chromatium*, strain D and in *Rhodopseudomonas spheroides*. Isolation of both species has been performed by variation in culture medium, and growth under different culture conditions has been investigated.

Direct correlation between carotenoid deficiency and changes in the near infrared spectrum of *Chromatium* could not be established. The appearance of

a 'stair-case type' of absorption spectrum in the presence of diphenylamine is found only when other conditions, e.g., low light intensity render this possible.

Carbonate instead of malate in the medium gave rise to a less pronounced stair-case type of spectrum.

In *Rhodopseudomonas* the height of the 850 mμ absorption peak is neither found to decrease in the presence of diphenylamine nor at low light intensities, as it is in *Chromatium*. The relation in height of the two main absorption peaks (E850/E795) in *Rhodopseudomonas* is sometimes even increased.

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