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Mutants of *Rhodospirillum rubrum* Obtained After Long-Term Anaerobic, Dark Growth

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Rhodospirillum rubrum S_1 cells were grown for more than 100 generations under strict anaerobic, dark conditions in liquid medium with sodium pyruvate. During this time, growth became nonpigmented. When cells were streaked onto the surface of solid growth medium in anaerobic bottles and placed in the dark, a few light-red colonies developed, but the majority was nonpigmented. Mutants were obtained from colonies selected on the basis of pigmentation and bacteriochlorophyll *a* content. The growth, ultrastructure, and light reactivity of two mutants were examined. Mutant C synthesized bacteriochlorophyll *a* (7.2 μmoles per mg of protein), altered membrane structures, and chromatophores during dark growth. Examination of light-induced changes of the absorption spectrum of this mutant suggested that only an electron transport pathway, which included the low potential cytochrome-like pigment C428, could be detected. Mutant C grew anaerobically in the light, whereas mutant G1 was light sensitive and produced only trace amounts of bacteriochlorophyll *a* (0.6 μmole per ml of protein). Poorly pigmented G1 cells contained unusual membrane structures. When dark-grown G1 colonies were placed in the light, deep-red colored papillae developed in the nonpigmented colonies. During anaerobic, dark growth with sodium pyruvate, both C and G1 synthesized poly-β-hydroxybutyrate and produced acetate, carbon dioxide, and hydrogen gas.

We reported recently that light was not required for the growth of purple nonsulfur bacteria under strict anaerobic conditions (32); when anaerobic, light-grown *Rhodospirillum rubrum* S_1 cells were placed in a dark environment, growth proceeded with no significant lag in protein synthesis. During 10 to 15 generations in the dark, pigments and associated membrane structures were synthesized which appeared to be functionally and structurally similar to those required for photosynthetic metabolism. Based upon these observations, it was suggested that in nature the retention of a preformed photosynthetic apparatus would be an advantage for the cell in a transitory anaerobic, dark environment.

The present study is the first investigation of structural and functional changes in photosynthetic purple nonsulfur bacteria after long-term anaerobic, dark growth.

MATERIALS AND METHODS

Organism, medium, and growth conditions. *R. rubrum* S_1 was kindly supplied by H. Gest. Cells were grown at 37 C in a complex medium which contained sodium pyruvate as previously described (32). Sodium phosphate buffer (*p*H 7.0) was sterilized and added separately to liquid medium to produce a final concentration of 0.1 M. For anaerobic growth, medium was prepared under an argon atmosphere by a modification of the Hungate technique (3, 16). Light or dark growth conditions were achieved as previously described (32).

Selection of mutant strains. Cells grown for 24 hr in dark conditions were spread over the surface of solid growth medium in anaerobic bottles (32); certain bottles were placed in light and others in dark conditions. Cells were obtained from isolated colonies selected on the basis of pigmentation and were subcultured twice under the same growth conditions on solid medium before preparation of stock cultures. Mutant cells remained viable after 1 year when placed in glycerol solution (1 ml per 100 ml of bacterial culture) and maintained at −70 C.

Determination of cell numbers. An anaerobic bottle was inoculated by spreading a 0.1-ml sample from a serial dilution of a culture onto the surface of solid growth medium; viable cell numbers were determined by colony count. Cells were counted directly with a Petroff-Hauser bacterial cell counting chamber.

Bacteriochlorophyll. Cells grown in liquid medium were collected by centrifugation and washed in distilled water. Bacteriochlorophyll *a* was extracted as pre-

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viously described (32), and the concentration was determined spectrophotometrically by use of the molar extinction coefficient (ϵ = 75 mM^{-1} cm^{-1}) at 770 nm by the method of Clayton (5).

Protein. Samples were digested with 1 M NaOH at room temperature for 24 hr. Protein content was estimated by the method of Lowry et al. (20). Bovine serum albumin was used as a standard.

Gas chromatography. Hydrogen and carbon dioxide were detected with a Packard gas chromatograph equipped with a silica gel column connected to an argon ionization detector (32). Volatile fatty acids were determined with a gas chromatograph by the method of Erwin, Marco, and Emery (9).

Poly-β-hydroxybutyric acid. Cells were hydrolyzed with 5.25% sodium hypochlorite or Clorox, and poly-β-hydroxybutyric acid (PHB) was extracted from the residue with hot chloroform by the method of Law and Slepecky (19). The extracted PHB was hydrolyzed with concentrated sulfuric acid, and the concentration was calculated from the molar extinction coefficient at 235 nm.

Spectroscopy. The characterization of bacteriochlorophyll *a* and carotenoids was determined with a Cary 14 recording spectrophotometer with whole cell suspensions in 60% sucrose (24).

Light-induced changes in absorbance were measured in a split-beam absorption difference spectrophotometer described earlier (28). For these measurements, whole cells were collected by low-speed centrifugation and suspended in a salts solution (*p*H 6.8) which contained no organic substrates (28).

Photomicroscopy. Photographs of colonies were taken with a Zeiss photomicroscope equipped with a 1× objective lens.

Electron microscopy. Cells grown for 24 to 36 hr under anaerobic, dark conditions were fixed by a modification of the method of Kellenberger, Ryter, and Séchaud (17). A 1% (w/v) solution of osmium tetroxide at *p*H 7.0 was used. Agar blocks were dehydrated, and thin sections were prepared and stained as previously described (32). Sections were examined with a Siemens Elmiskop 1 electron microscope operating at 60 kv, equipped with a 50-μm objective aperture unless otherwise indicated.

RESULTS

R. rubrum cells, maintained for more than 100 generations under anaerobic, dark growth conditions, remained scotophobic and the bacteriochlorophyll *a* concentration slowly decreased to levels of 0.3 to 0.5 μmole per mg of protein. [Interruption of the light for approximately 1 sec in a light microscope causes a phototrophic bacterial cell to reverse its directional movement. Contrary to the phototactic response in green plants, the bacterial response is oriented not toward a light source but rather away from darkness (8, 21, 23). For this reason we propose the term scotophobic response (Gr. *skotos* darkness + *phobos* fear). We suggest that scotophobic is an adjective which describes the specific response or "Schreckbewegung" (8) in the photosynthetic bacteria.] If cells were streaked onto the surface of solid growth medium in an anaerobic bottle and placed in the dark, colonies 2 to 3 mm in diameter formed that differed in pigmentation. Most of the colonies were white or faint pink; however, a few developed light-red pigmentation. Mutant strains C and G1 were obtained from colonies selected on the basis of pigmentation.

Colony pigmentation. Dark-grown mutant C developed light-red colonies. Cells were scotophobic and synthesized approximately 7.2 μmoles of bacteriochlorophyll *a* per mg of protein (Table 1). Similar values were obtained previously for cells grown anaerobically in the dark for 10 to 15 generations after transfer from light conditions (32). G1 cells formed white or slightly pink-colored colonies in the dark and synthesized trace amounts of bacteriochlorophyll *a* (Table 1). Poorly pigmented G1 cells were scotophobic during early logarithmic growth. No photosynthetic pigments were synthesized during aerobic, dark growth of C or G1 (Table 1), and a scotophobic response could not be demonstrated.

Response to light. Growth of mutants C and G1 in light conditions was determined by measuring increase in protein (Fig. 1). During the first 12 hr after transfer into light, the protein concentration decreased, perhaps a result of cell lysis. Cell protein in C, however, increased fivefold during the following 36 hr, and the ratio of bacteriochlorophyll *a* to protein increased to 16.2 (Table 1). Cell protein in G1 doubled and after 48 hr decreased as the cells lysed. No growth of G1 was detected during 4 additional days in the light. After 36 hr growth in the dark, G1 reached a density of 5×10^8 cells per ml.

A suspension of dark-grown mutant C was serially diluted under anaerobic conditions, and a sample of each dilution was spread onto the surface of solid growth medium in a separate anaerobic bottle. One set of anaerobic bottles, inoculated with each dilution, was placed in the light; a second set was placed in the dark. During 7 days in dark conditions, colonies 2 to 3 mm in diameter formed. Anaerobic bottles placed in the light were examined for colony formation, and an equal number of C colonies developed in light or dark conditions (Table 2, experiments 1 and 2). Under dark conditions C colonies were light red, and those formed in the light were dark red. When dark-grown C colonies were transferred into the light, pigmentation increased; if light-grown colonies were placed in the dark, no loss in pigmentation was observed. The number of colonies did not increase when anaerobic bottles were switched from dark to light conditions or vice versa.

TABLE 1. *Bacteriochlorophyll a produced by mutants of Rhodospirillum rubrum S_1 under different growth conditions in liquid medium*

Mutant strain	Growth condition	Period of incubation (hr)	Bacteriochlorophyll (μmoles)	Protein (mg)	Bacteriochlorophyll per mg of protein (μmoles)
C	Dark, anaerobic	33	20.80	2.44	8.1
		36	25.20	3.42	7.4
		38	14.02	2.26	6.2
C	Light,[a] anaerobic	53	13.44	0.83	16.2
		53	14.84	0.98	15.1
		53	16.00	0.89	18.0
C	Dark, aerobic	26	0.00	1.14	0.0
		36	0.00	1.28	0.0
		48	0.00	1.05	0.0
G1	Dark, anaerobic	36	1.79	3.02	0.6
		38	1.00	1.52	0.7
		38	0.48	1.42	0.3
G2[b]	Light, anaerobic	38	20.48	1.25	16.4
		46	20.43	1.19	17.2
		72	20.70	1.00	20.7
G1	Dark, aerobic	24	0.00	2.14	0.0
		25	0.00	1.12	0.0
		27	0.00	2.05	0.0

[a] Approximately 60 foot candles.
[b] Photosynthetic revertant obtained from light-sensitive G1.

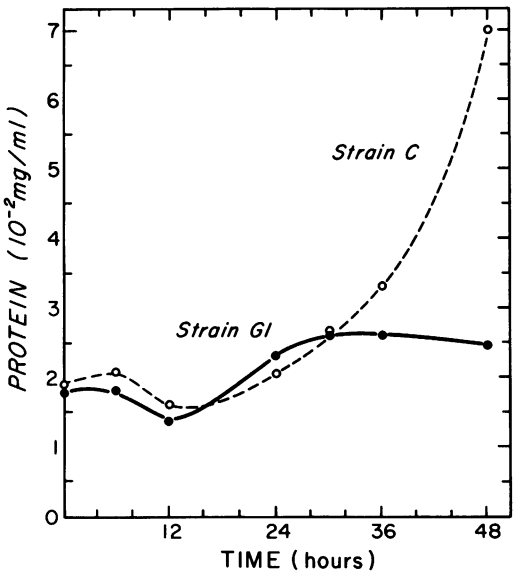

FIG. 1. *Increase of protein in Rhodospirillum rubrum S_1 mutant C (○) and G1 (●) transferred from anaerobic, dark into anaerobic, light (55 to 60 foot candles) conditions.*

When anaerobic bottles were inoculated (as described above) with dark-grown G1 and were placed in the light, only 2 to 3 out of 10^6 cells grew and formed deep-red pigmented colonies (Table 2, experiments 3 and 4). When these bottles were placed in the dark for 14 days, no additional colonies developed. Accordingly, when bottles containing nonpigmented, G1 dark-formed colonies were placed in the light, no increase in isolated colonies occurred. Under these conditions, however, deep-red pigmented papillae formed within or on the periphery of 80 to 90% of the colonies (Fig. 2). An average of one to two papillae developed per colony. In one case, however, this number reached as high as 11.

TABLE 2. *Growth of mutants of Rhodospirillum rubrum S_1 under anaerobic, dark or light conditions on solid medium*

Expt	Mutant strain	Growth condition[a]	Dilution	Colonies counted[b]	
				Pigmented	Nonpigmented
1	C	Dark	10^5	0	230
		Light[c]	10^5	280	0
2	C	Dark	10^6	0	100
		Light	10^6	70	0
3	G1	Dark	10^5	0	250
		Light	10^1	48	0
4	G1	Dark	10^6	0	59
		Light	Undiluted	156	0

[a] Colonies were allowed to develop for 9 days at 37 C under the respective growth condition.
[b] Colonies were assayed visually. Nonpigmented colonies were faint red (strain C) or faint pink-white (strain G1). Pigmented colonies were dark red.
[c] Approximately 60 foot candles.

Mutant G2 was obtained from a papilla which developed under these conditions. Photosynthesizing G2 cells were scotophobic and produced bacteriochlorophyll *a* at concentrations similar to those observed for light-grown C cells (Table 1).

Internal membrane structure. Mutant C produced a variety of internal structures during dark growth. Chromatophores were formed which appeared similar to those in light-grown cells. Figure 3 is an electron micrograph of a thin longitudinal section of a dark-grown cell in which chromatophores were aligned along the cell membrane. In a few sections they extended throughout the cytoplasm; in others, they were absent. Thin sections of light-grown C were filled with chromatophores and appeared similar to G2 (see Fig. 9) and to normal *R. rubrum* S_1 (32).

Figure 4 is a thin transverse section through a complex membrane structure; these structures were observed frequently in dark-grown C. This structure was composed of a series of concentric membrane vesicles. Each vesicle enclosed an electron-translucent area with a density similar to that inside chromatophores (see Fig. 9). Cytoplasmic material appeared to be deposited in the center of the structure and in the spaces between the encircling vesicles. Similar membrane configurations have been observed in *R. rubrum* (26) and *Rhodopseudomonas palustris* (30, 31) placed under different conditions. In *R. palustris* they appeared between the cytoplasmic membrane and the cell wall and were suggested to represent flagellar basal bodies (30, 31). This, however, does not seem likely in C (6). Concentric membrane structures have not been observed in G1.

Internal vesicles composed of a unit membrane that contained an electron-dense material were observed in dark-grown C (Fig. 5) and G1. The vesicles remained attached to internal membrane material in lysed cell preparations (Fig. 6 and 7), together with chromatophores, and probably developed from membrane invaginations. Schmidt and Kamen (25) noted granules with similar staining properties in *Chromatium* strain D cells. These granules, however, were not surrounded by a unit membrane and probably represented glycogen deposits. The function and chemical composition of the electron-dense vesicles in *R. rubrum* is not known.

The internal structure of G1 was different from C; G1 synthesized only trace amounts of photosynthetic membrane, and a typical chromatophore was rarely observed in thin sections (Fig. 8). On the other hand, mutant G2, a photosynthetic revertant, was filled with chromatophores (Fig. 9) and appeared similar to wild-type cells (32). G1 cells contained electron-dense

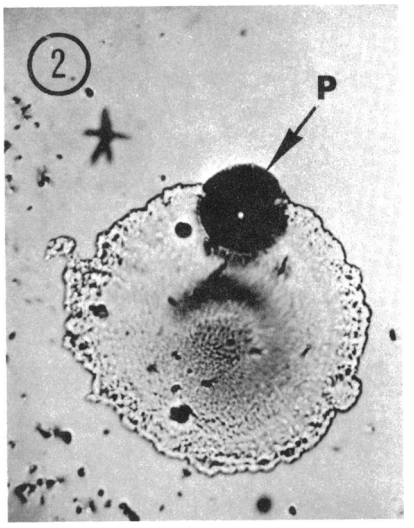

FIG. 2. *Rhodospirillum rubrum* S_1 *G1 nonpigmented colony formed during anaerobic, dark growth containing a pigmented papilla (P) which developed after transfer into light.* ×10.

areas of myelin-like material, or membrane aggregates, which extended through the cytoplasm in an unordered manner. The structures appeared to be formed from unit membranes (Fig. 10), approximately 9 nm across, that were continuous with the cytoplasmic membrane (Fig. 11). Similar structures have been observed in dark-grown C. Myelin-like deposits have been reported in *R. rubrum* maintained under different conditions (15, 26). Schoen and Ladwig (26) suggested that these deposits resulted from the deterioration of chromatophores. Membrane aggregates in G1 cells are probably formed by invaginations of the cytoplasmic membrane, independent from chromatophore formation. The function of these structures and their relationship to photosynthetic membranes is not known. Similar membranous configurations have been reported in nonphotosynthesizing cells such as *Vibrio marinus* (10) and *Escherichia coli* (33).

Light absorption. Dark-grown mutant C demonstrated an absorption spectrum similar to light-grown *R. rubrum* (32). To observe the spectrophotometric absorption properties of pigments in mutant G1, it was necessary to use a sensitive instrumental range of 0.0 to 0.2 optical density units. Cells grown under aerobic, dark conditions, where no photosynthetic pigments were synthesized (Table 1), were suspended in sucrose and placed in the reference beam. Anaerobic-grown G1 cells, observed under these conditions, demonstrated absorption maxima for bacteriochlorophyll *a* at 890 and 806 nm with a light

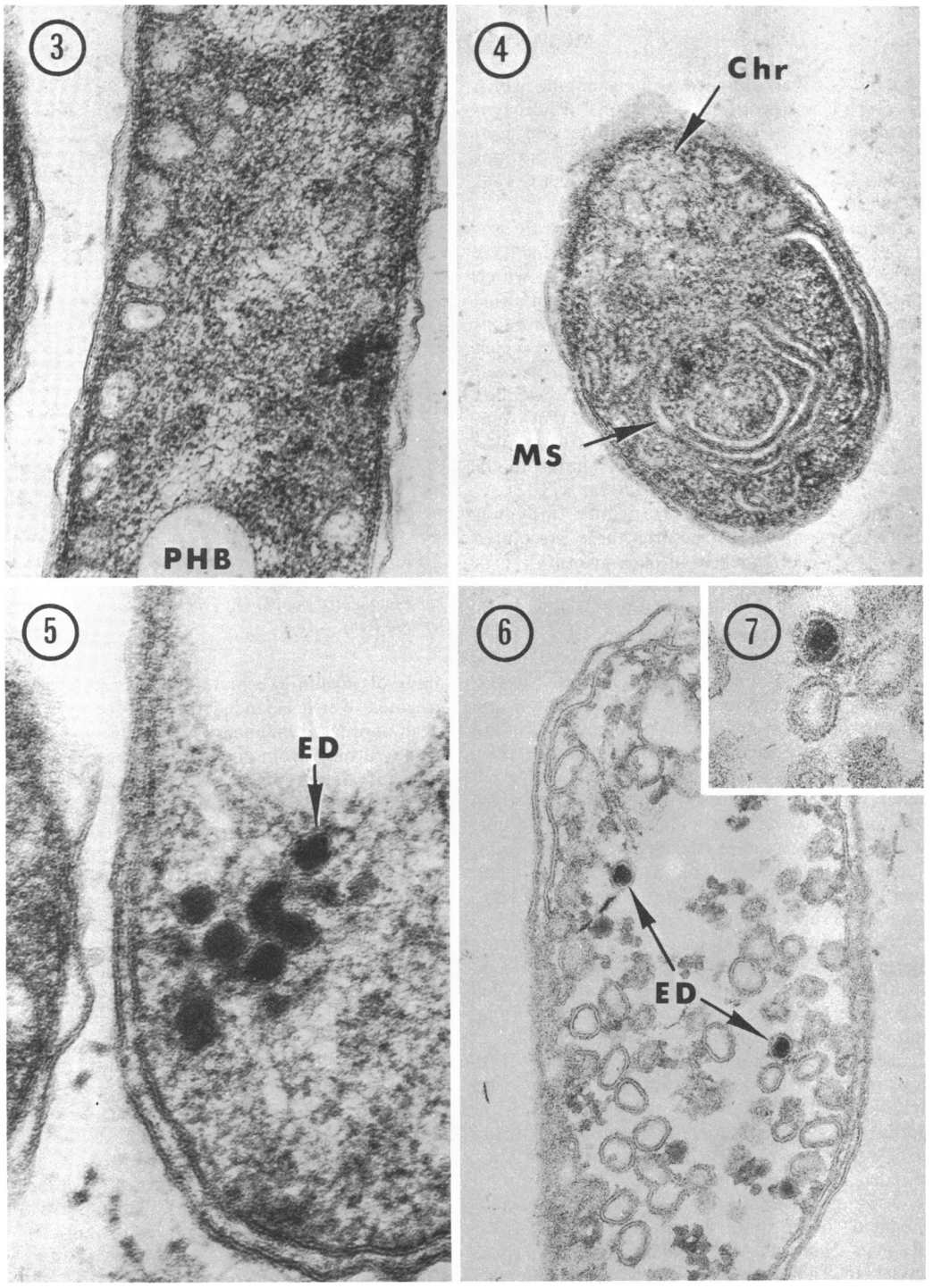

FIG. 3. *Electron micrograph of longitudinal thin section of anaerobic, dark-grown mutant C that contained chromatophores and a poly-β-hydroxybutyrate (PHB) storage granule. ×88,000.*

FIG. 4. *Electron micrograph of thin section through anaerobic, dark-grown mutant C showing a concentrically aligned membrane structure (MS) and chromatophores (Chr). ×80,000.*

FIG. 5. *Electron micrograph of thin section of anaerobic, dark-grown mutant C containing membrane-bound, electron-dense vesicles (ED). ×144,000.*

FIG. 6. *Electron micrograph of thin section through vesicles (ED) attached to membrane material inside mutant C lysed by osmotic shock treatment (22). Cells were fixed with glutaraldehyde (3% w/v) followed by osmium tetroxide. (Phillips 300 EM electron microscope operating at 80 kv, 30-μm objective aperture.) ×82,000.*

FIG. 7. *Enlargement of the vesicle in the lower right portion of Fig. 6. ×164,000.*

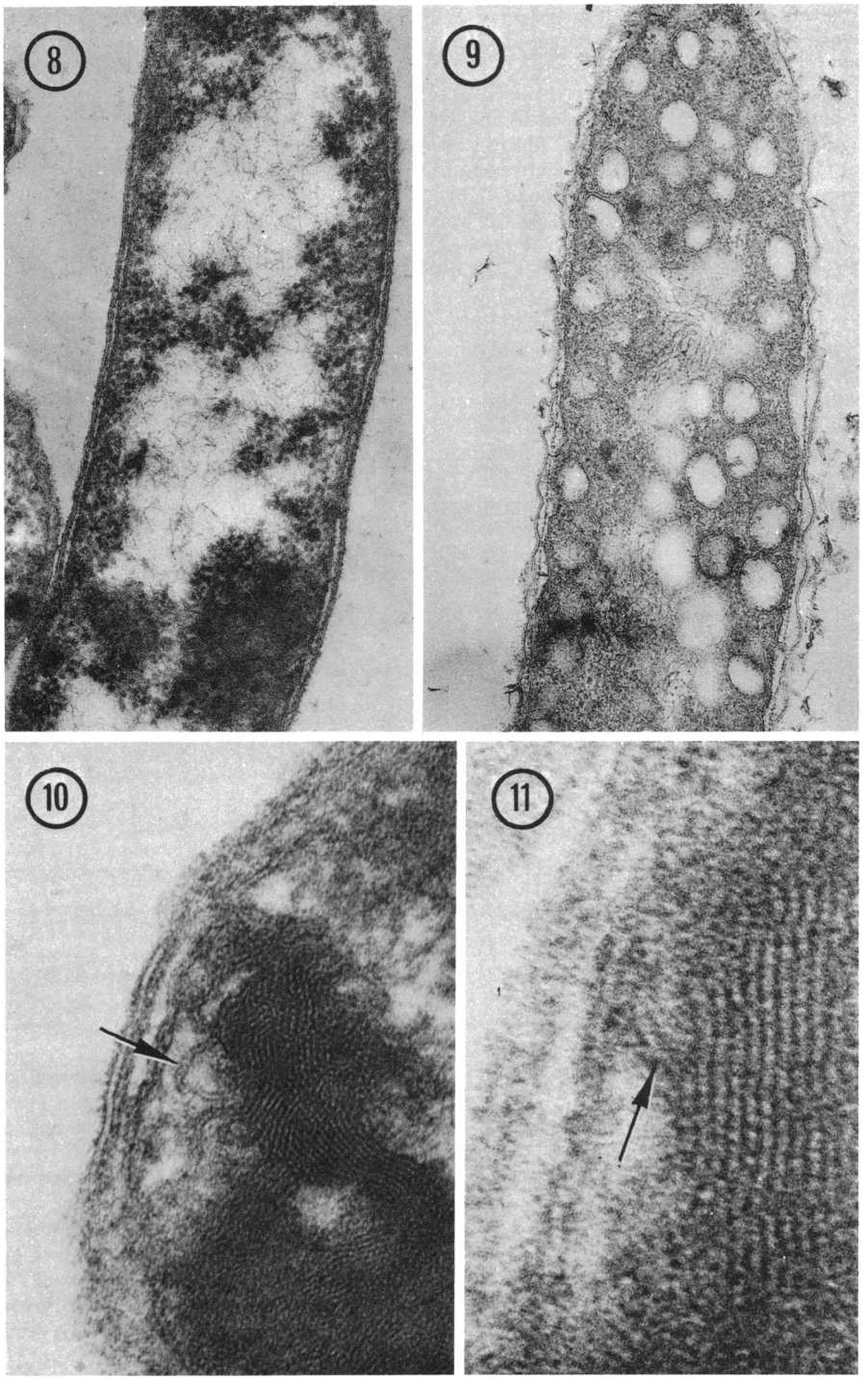

FIG. 8. *Electron micrograph of thin longitudinal section of anaerobic, dark-grown mutant G1.* ×*60,000.*

FIG. 9. *Electron micrograph of thin longitudinal section of anerobic, light-grown revertant G2.* ×*64,000.*

FIG. 10. *Electron micrograph of thin section through a membrane aggregate observed in anaerobic, dark-grown mutant G1. Loops of membrane (arrow) extend into the surrounding cytoplasm.* ×*144,000.*

FIG. 11. *Electron micrograph of thin section of anaerobic, dark-grown mutant G1 showing a membrane aggregate continuous with (arrow) the cell membrane (operating at 80 kv, 35-μm objective aperature).* ×*440,000.*

absorption ratio of approximately 2:1, respectively. Only trace amounts of carotenoids were detectable.

Light-induced reactions. Photosynthetic activity of both G1 and C was examined by measuring light-induced changes in the absorption spectrum. In G1 cells, no light-induced reaction of cytochromes could be detected. Small light-induced changes in the near-infrared spectral region, however, indicated some photo-oxidation of reaction center bacteriochlorophyll *a*. The light-minus-dark difference spectrum (Fig. 12) showed a bleaching centered at about 870 nm and a blue shift of an absorption band at about 800 nm. These spectral features are characteristic for the photo-oxidation of the high-potential (+440 mv) reaction center P870 (4). The extent of the absorption change was small, and the reaction was sluggish.

In contrast, C cells showed marked and much more efficient photosynthetic activity. The photo-oxidation of only one cytochrome, even at actinic intensities as high as 7 nano-Einstein per cm^2 per sec, could be detected. Figure 13 shows that this cytochrome is the low-potential, cytochrome-like pigment C428, which was previously found to be photo-oxidized at low actinic light intensities (28). Mutant C did not show absorbance changes characteristic for the oxidation of P870 photoreaction center. The light-minus-dark spectrum (Fig. 14) in the near-infrared spectral region showed features which could be interpreted as a red shift of an absorption band at about 865 nm (11, 14). The light-induced absorbance changes in the near-infrared spectral region were rapid and monophasic and were the only ones measured in dark-grown C in the near-infrared spectral region.

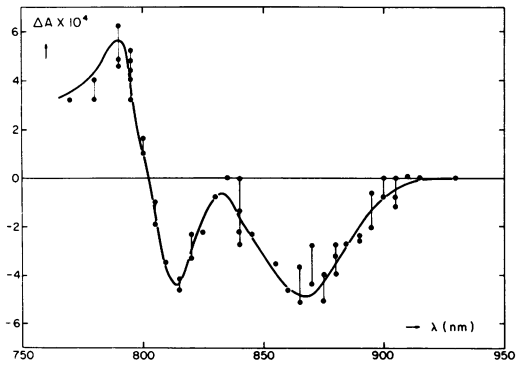

FIG. 12. *Light-minus-dark difference spectrum of absorbance changes in mutant G1. Intensity of actinic light at 586 nm was 3.5 nano-Einstein per cm^2 per sec. Whole cell suspension; approximately 0.6 μmole of bacteriochlorophyll a per mg of protein.*

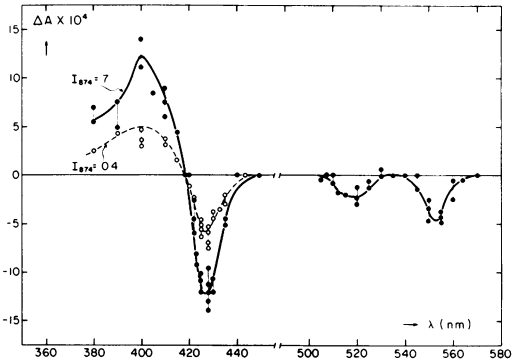

FIG. 13. *Light-minus-dark difference spectra of absorbance changes in the visible spectral region of mutant C. Actinic light at 874 nm (I_{874}) was at intensities of 0.4 (○) and 7 (●) nano-Einstein per cm^2 per sec. Whole cell suspension; approximately 8.0 μmoles of bacteriochlorophyll a per mg of protein.*

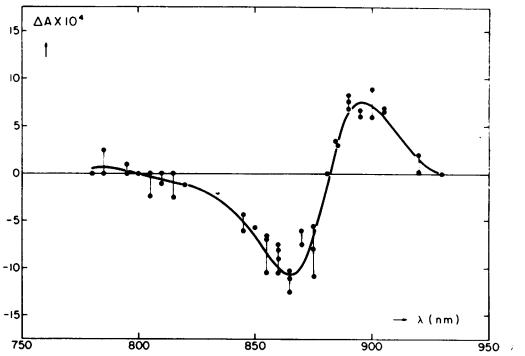

FIG. 14. *Light-minus-dark difference spectrum of absorbance changes in the near-infrared spectral region in mutant C. Intensity of actinic light at 586 nm was 2.4 nano-Einstein per cm^2 per sec. Whole cell suspension; approximately 8.0 μmoles of bacteriochlorophyll a per mg of protein.*

Metabolism of pyruvate. *R. rubrum* grew rapidly in the dark under an argon atmosphere in the presence of sodium pyruvate and produced acetate, carbon dioxide, and hydrogen gas. In addition, cells synthesized poly-β-hydroxybutyric acid storage granules (Fig. 3). The synthesis of this product is presented as a function of cell protein in Table 3. After 65 hr of dark growth, C cells contained approximately twice as much poly-β-hydroxybutyric acid as cells grown in the light for 72 hr. Light-sensitive G1 accumulated almost four times as much poly-β-hydroxybutyric acid after 24 hr of growth than did photosynthesizing G2 cells after 72 hr. The metabolism of sodium pyruvate and synthesis of poly-β-hydroxybutyrate during dark growth is being investigated.

TABLE 3. *Poly-β-hydroxybutyric acid (PHB) production by mutants of Rhodospirillum rubrum S_1 under anaerobic, dark or light growth conditions in liquid medium*

Mutant strain	Growth condition[a]	Growth period (hr)	PHB (μmoles)	Protein (mg)	PHB per mg of protein (μmole)
C	dark	24	0.53	2.44	0.22
		48	0.74	1.80	0.41
		65	0.84	1.42	0.58
C	light[b]	48	0.25	1.32	0.19
		72	0.82	2.88	0.28
G1	dark	24	2.13	2.60	0.82
		32	1.84	2.00	0.92
G2	light	48	0.27	3.60	0.08
		72	0.80	3.87	0.21

[a] Cells grown under strict anaerobic conditions.
[b] Approximately 60 foot candles.

It was noted that cells produced hydrogen sulfide during dark growth. Cystein, supplied as a reducing agent in the crude growth medium (3, 32), may be metabolized to pyruvate with the release of H_2S.

DISCUSSION

In these initial studies on cells which have been transferred and grown for long periods of time anaerobically in the dark, the amount of bacteriochlorophyll *a* synthesized during growth gradually decreased, and after more than 100 generations only trace amounts were detected. Observations of thin sections of these cells demonstrated two general types of internal structure: strain C, which formed chromatophores, and strain G1, in which chromatophores were difficult to find in thin sections.

The function of a photosynthetic apparatus during light growth has been extensively studied; it is now possible to study its activity during anaerobic, dark growth. The photosynthetic apparatus present in C appears different from normal cells. Characteristics of cyclic light-induced electron transport (the photo-oxidation of the reaction center bacteriochlorophyll P870 and cytochrome c_2) are missing (cf. reference 29). Only the low-potential pigment C428, which has been proposed to function during noncyclic electron transport (14, 28), is oxidized by light (Fig. 13). No indication of light-induced oxidation of reaction center bacteriochlorophyll P870 is seen. Instead, light-induced absorbance changes in the near-infrared spectral region showed a red shift of an absorption band at about 865 nm (Fig. 14). Similar reactions are present in normal photosynthesizing cells in a late stage of development (11, 14). The light-induced, near-infrared absorbance changes may reflect the photo-oxidation of a low-potential reaction center component, different from P870, which is re-reduced by the subsequent oxidation of pigment C428. The presence of only pigment C428 and a reaction center component different from P870 may mean that mutant C synthesizes a low-potential electron transport system which can function during dark metabolism. Pigment-producing cells that develop during long-term dark growth in low redox conditions may be selected for their ability to repress synthesis of the high-potential cyclic electron transport pathway required in photophosphorylation (4, 29). In the light, P870 becomes functional during photosynthetic growth. The absence of P870 reaction center in these dark-grown cells cannot be stated with certainty, however. It is possible that the reaction between C428 and high-potential P870 is so fast that it cannot be observed in experiments with continuous actinic light even at high intensities; flashing light should give results which are conclusive in this respect.

In poorly pigmented G1, no light-induced cytochrome reactions could be detected. The light-induced absorbance changes measured in the near-infrared spectral region (Fig. 12) show a reversible photo-oxidation of high-potential P870 reaction center component. The absorbance changes, however, were small and sluggish. Because of this, the lack of cytochrome activity, and the discovery that G1 is light-sensitive, the rudimentary remains of a P870 photoreactive center may not be physiologically functional. The transport of electrons in these nonpigmented cells is probably different from the noncyclic photosynthetic mechanism suggested for C.

The production of poly-β-hydroxybutyric acid and hydrogen during dark fermentation of pyruvate is difficult to reconcile with published data (1, 2, 12, 13, 18, 27; K. T. Shanmugam and D. I. Arnon, Fed. Proc., p. 1136, 1971). To our knowledge, this is the first report of poly-β-hydroxybutyric acid synthesis in fermentative me-

tabolism. The data suggest that an oxidative pyruvate clastic reaction may function during dark growth, electrons being transferred to hydrogenase as well as to the polymer-forming system (2, 7, 27).

Continued study of purple nonsulfur bacteria during anaerobic, dark growth may provide solutions to a few of the unresolved questions regarding the metabolic activities of this versatile group of microorganisms. Hopefully, use of these strict anaerobic, dark growth techniques presents additional opportunities to obtain mutant cells which will contribute to our understanding of the control and function of the photosynthetic apparatus in light-grown cells. The observation that *R. rubrum* becomes diverse under prolonged anaerobic, dark growth (in the absence of the selective pressure of radiant energy) suggests that a similar process could occur as well in a natural aquatic environment.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. Bennett, R., N. Rigopoulos, and R. C. Fuller. 1964. The pyruvate phosphoroclastic reaction and light-dependent nitrogen fixation in bacterial photosynthesis. Proc. Nat. Acad. Sci. U.S.A. **52**:762-768.
2. Bosshard-Heer, E., and R. Bachofen. 1969. Synthese von Speicherstoffen aus Pyruvat durch *Rhodospirillum rubrum*. Arch. Mikrobiol. **65**:61-75.
3. Bryant, M. P., and I. M. Robinson. 1961. An improved nonselective culture medium for ruminal bacteria and its use in determining diurnal variation in numbers of bacteria in the rumen. J. Dairy Sci. **44**:1446-1456.
4. Clayton, R. K. 1962. Primary reactions in bacterial photosynthesis. I. The nature of light-induced absorbancy changes in chromatophores: evidence for a special bacteriochlorophyll component. Photochem. Photobiol. **1**: 201-210.
5. Clayton, R. K. 1963. Toward the isolation of a photochemical reaction center in *Rhodopseudomonas spheroides*. Biochim. Biophys. Acta **75**:312-323.
6. Cohen-Bazire, G., and J. London. 1967. Basal organelles of bacterial flagella. J. Bacteriol. **94**:458-465.
7. Dawes, E. A., and D. W. Ribbons. 1964. Some aspects of the endogenous metabolism of bacteria. Bacteriol. Rev. **28**:126-149.
8. Engelmann, T. W. 1883. *Bacterium photometricum*. Ein Beitrag zur vergleichenden Physiologie des Licht- und Farbensinnes. Pfleugers Arch. Gesamte Physiol. Menschen Tiere **30**:95-128.
9. Erwin, E. S., G. B. Marco, and E. M. Emery. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci. **44**:1768-1771.
10. Felter, R. A., S. F. Kennedy, R. R. Colwell, and G. B. Chapman. 1970. Intracytoplasmic membrane structures in *Vibrio marinus*. J. Bacteriol. **102**:552-560.
11. Fowler, C. F., and C. Sybesma. 1970. Light- and chemically-induced oxidation-reduction reactions in chromatophore fractions of *Rhodospirillum rubrum*. Biochim. Biophys. Acta **197**:276-283.
12. Gest, H. 1952. Properties of cell-free hydrogenases of *Escherichia coli* and *Rhodospirillum rubrum*. J. Bacteriol. **63**:111-121.
13. Gest, H., J. G. Ormerod, and K. S. Ormerod. 1962. Photometabolism of *Rhodospirillum rubrum:* light-dependent dissimilation of organic compounds to carbon dioxide and molecular hydrogen by an anaerobic citric acid cycle. Arch. Biochem. Biophys. **97**:21-33.
14. Govindjee, R., and C. Sybesma. 1970. Light-induced reduction of pyridine nucleotide and its relation to light-induced electron transport in whole cells of *Rhodospirillum rubrum*. Biochim. Biophys. Acta **223**:251-260.
15. Hickman, D. D., and A. W. Frenkel. 1959. The structure of *Rhodospirillum rubrum*. J. Cell Biol. **6**:277-284.
16. Hungate, R. E. 1950. The anaerobic mesophilic cellulolytic bacteria. Bacteriol. Rev. **14**:1-49.
17. Kellenberger, E., A. Ryter, and J. Séchaud. 1958. Electron microscope study of DNA-containing plasms. II. Vegetative and mature phage DNA compared with normal bacterial nucleoids in different physiological states. J. Cell Biol. **4**:671-675.
18. Kohlmiller, E. F., Jr., and H. Gest. 1951. A comparative study of the light and dark fermentations of organic acids by *Rhodospirillum rubrum*. J. Bacteriol. **61**:269-282.
19. Law, J. H., and R. A. Slepecky. 1961. Assay of poly-β-hydroxybutyric acid. J. Bacteriol. **82**:33-36.
20. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
21. Manten, A. 1948. Phototaxis in the purple bacterium *Rhodospirillum rubrum*, and the relation between phototaxis and photosynthesis. Antonie van Leeuwenhoek J. Microbiol. Serol. **14**:65-86.
22. Oppenheim, J., and L. Marcus. 1970. Correlation of ultrastructure in *Azotobacter vinelandii* with nitrogen source for growth. J. Bacteriol. **101**:286-291.
23. Pfennig, N. 1967. Photosynthetic bacteria, p. 285-324. *In* C. E. Clifton, S. Raffel, and M. P. Starr (ed.), Annual review of microbiology, vol. 21. Annual Reviews, Inc., Palo Alto, Calif.
24. Pfennig, N. 1969. *Rhodopseudomonas acidophila*, sp. n., a new species of the budding purple nonsulfur bacteria. J. Bacteriol. **99**:597-602.
25. Schmidt, G. L., and M. D. Kamen. 1970. Variable cellular composition of *Chromatium* in growing cultures. Arch. Mikrobiol. **73**:1-18.
26. Schoen, G., and R. Ladwig. 1970. Bacteriochlorophyllsynthese und Thylakoidmorphogenese in anaerober Dunkelkulture von *Rhodospirillum rubrum*. Archiv. Mikrobiol. **74**:356-371.
27. Stanier, R. Y., M. Doudoroff, R. Kunisawa, and R. Contopoulou. 1959. The role of organic substrates in bacterial photosynthesis. Proc. Nat. Acad. Sci. U.S.A. **45**: 1246-1260.
28. Sybesma, C., and C. F. Fowler. 1968. Evidence for two light-driven reactions in the purple photosynthetic bacterium, *Rhodospirillum rubrum*. Proc. Nat. Acad. Sci. U.S.A. **61**:1343-1348.
29. Sybesma, C. 1970. Photosynthetic bacteria, p. 57-93. *In* P. Halldall (ed.), Photobiology of microorganisms. John Wiley & Sons Inc., New York.
30. Tauschel, H.-D., and G. Drews. 1969. Der Geisselapparat von *Rhodopseudomonas palustris*. II. Entstehung und Feinstruktur der Geissel-Basalkörper. Archiv Mikrobiol. **66**:180-194.
31. Tauschel, H.-D., and G. Drews. 1969. Zum Vorkommen helixförmig angeordneter Ribosomen bei *Rhodopseudomonas palustris*. Archiv Mikrobiol. **64**:377-386.
32. Uffen, R. L., and R. S. Wolfe. 1970. Anaerobic growth of purple nonsulfur bacteria under dark conditions. J. Bacteriol. **104**:462-472.
33. Weigand, R. A., J. M. Shively, and J. W. Greenawalt. 1970. Formation and ultrastructure of extra membranes in *Escherichia coli*. J. Bacteriol. **102**:240-249.