*SOME OBSERVATIONS ON THE SYNTHESIS AND FUNCTION OF THE PHOTOSYNTHETIC APPARATUS IN RHODOSPIRILLUM RUBRUM**

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The chlorophyll content of nonsulfur purple bacteria varies greatly in response to environmental factors, of which the most important are light intensity and oxygen.[1] In cells growing anaerobically in the light (i.e., under conditions of obligatory photosynthesis), the differential rate of chlorophyll synthesis is an inverse function of light intensity. Many nonsulfur purple bacteria can also grow aerobically, at the expense of respiratory metabolism. Oxygen inhibits chlorophyll synthesis, and accordingly cells of such purple bacteria grown under stringent aerobic conditions, whether in the dark or in the light, are devoid of chlorophyll. Chlorophyll synthesis can, however, take place even in the dark at very low oxygen tensions. Consequently cultures grown with a restricted oxygen supply contain considerable amounts of pigment. The observations concerning environmentally conditioned changes in the chlorophyll content of purple bacteria have so far all been made on intact cells.

In bacteria, the photosynthetic pigment system can be isolated from disrupted cells in association with macromolecular particles known as chromatophores.[2] These particles are chemically complex, and contain as major constituents both protein and phospholipid, in addition to the photosynthetic pigments.[3, 4] They are the site of the primary photochemical reaction of bacterial photosynthesis, cyclic photophosphorylation.[5–7]

It is not clear whether gross changes in cellular chlorophyll content simply reflect fluctuations in the rate of chlorophyll synthesis and incorporation into an otherwise constant chromatophore structure, or whether they are one manifestation of much more complex, environmentally induced, changes in the biosynthesis, composition, and function of the whole chromatophore system.

This question can be answered only by the isolation of purified chromatophores from cells with different chlorophyll contents, followed by the systematic comparison of the various properties of such chromatophores. The present paper describes the influence of environmental factors that affect the chlorophyll content of whole cells on the specific chlorophyll content, the photophosphorylative capacity, and the succinic dehydrogenase activity of isolated chromatophores. The two enzymatic activities mentioned have both been shown to be associated with the chromatophores of purple bacteria.[6, 8] The former activity is clearly dependent on the presence of a photochemically functional pigment system, whereas the latter is not. Comparative measurements of these two activities could therefore be expected to reveal any differences between photochemically linked and strictly "dark" enzymatic functions which might occur in chromatophores prepared from cells grown under different conditions.

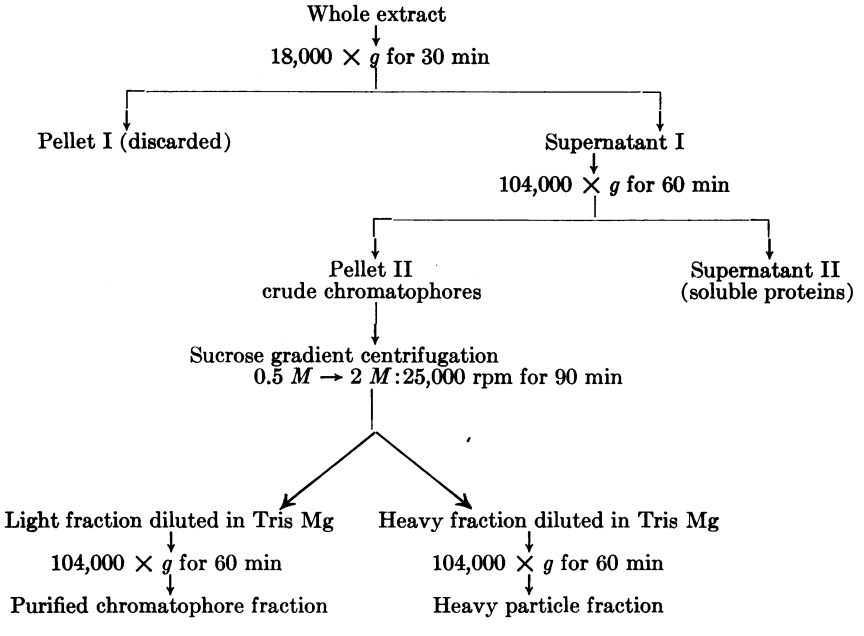
Materials and Methods.—Bacterial cultivation: Rhodospirillum rubrum, strain 1.1.1., from the collection of Professor C. B. van Niel was used in all experiments. Photosynthetic cultures were grown in the modified medium of Hutner,[1] with

malate as carbon source. The composition of the medium and the technical details of cultivation have already been described.[1, 9] Aerobic depigmented cultures were grown in the complete absence of light with mechanical agitation in Fernbach flasks (2.5-liter capacity), containing 500 ml of Hutner medium with succinate as carbon source. The inoculum consisted of depigmented cells, grown in the dark with strong aeration. Cultures were harvested during exponential growth, at a population density of less than 2×10^8 bacteria per ml. All cultures were grown at 30°C.

Measurements on cell suspensions and extracts: Cell mass was determined by measuring the optical density of cultures at 680 mμ in a Beckman DU spectrophotometer. The bacteriochlorophyll content of cell suspensions or cellular fractions was determined spectrophotometrically.[1] The Folin-Lowry method[10] was

TABLE 1

PROCEDURE USED FOR THE ISOLATION OF PURIFIED CHROMATOPHORES FROM EXTRACTS OF *Rhodospirillum rubrum*



used to measure the protein content of cell extracts and of cellular fractions. At the dilutions used for protein determination, the photosynthetic pigments, if present, do not interfere with the analysis. The protein content of intact cells of *R. rubrum* cannot be satisfactorily determined by either the Biuret or the Folin-Lowry method. In order to use the Biuret method, photosynthetic pigments must first be extracted with acetone-methanol, which also removes alcohol-soluble proteins. The Folin-Lowry method could in principle be used after alkaline digestion; however, the cells of *R. rubrum* are not digested completely in *N* NaOH, and the values for protein are low and not reproducible. When it was necessary to estimate the protein content of a cell suspension, the cells were first broken, and the estimation was made on the whole extract; this gave consistent and reproducible results.

Preparation and purification of bacterial chromatophores: Cells were harvested by centrifugation at 4°C, washed, and resuspended in chilled phosphate buffer (0.02 *M*, pH 7) containing 0.01 *M* $MgSO_4$. All subsequent operations were performed at 4°C. The concentrated suspension of cells in buffer was disintegrated in the French Pressure Cell (Aminco) under a pressure of 20,000 lb per square inch. The whole extract was then fractionated by the procedure outlined in Table 1. The first centrifugation at 18,000 × *g* for 30 min eliminated the whole cells and coarse debris and gave a transparent, pigmented supernatant which was subsequently centrifuged for one hour in a Spinco preparative centrifuge at 104,000 × *g*. The orange-pink supernatant liquid, containing the soluble proteins and large amounts of cytochrome c_2[11], was discarded. The pellet constituted the *crude chromatophore fraction.* The wall of the centrifuge tube as well as the pellet were rinsed with Tris-HCl buffer (0.33 *M*, pH 7.6) containing 0.01 *M* $MgSO_4$ (Tris-Mg buffer). The pellet of crude chromatophores was resuspended in a small volume of Tris-Mg buffer and the suspension homogenized with a hypodermic syringe. Part of the crude chromatophore suspension was kept for tests of enzymatic activity and analysis of pigment and protein content. The remainder was centrifuged through a linear sucrose density gradient in the swinging bucket rotor S W 25–1 of the preparative Spinco centrifuge, operated at 25,000 rpm for 90 min. The linear density gradient was established beforehand, in centrifuge tubes lightly coated with silicone grease. The mixing device of Bock and Ling[12] permitted the establishment of a linear gradient, using equal volumes of two sucrose solutions of different molarities.[13] We used routinely 11 ml of 2 *M* sucrose and 11 ml of 0.5 *M* sucrose to establish the gradient. The sucrose solutions were prepared in Tris-Mg buffer. Another linear sucrose gradient, using 2 ml of 0.4 *M* sucrose and 2 ml of the crude chromatophore suspension to be analyzed, was layered on top of the first gradient with a smaller mixing device. An equally satisfactory procedure is to layer with a slow flowing pipette on top of the first gradient a relatively small volume (1.5 ml) of a concentrated crude chromatophore suspension in Tris-Mg buffer or in 0.2 *M* sucrose.

After centrifugation at 25,000 rpm for 90 min, the gradient shows two main pigmented bands. The bottom one scatters light strongly and has a gelatinous consistency; it contains what we call the "heavy particle fraction." The upper band is pigmented and does not scatter light appreciably; we call the material in this band the "purified chromatophore fraction." The position of the middle of the purified chromatophore band (upper band) in the gradient corresponds to a concentration of sucrose of approximately 1.08 *M*. The lower pigmented band corresponds to a sucrose concentration varying between 1.77 and 1.8 *M*. It was not in general possible to collect the fractions directly by elution; most of the heavy fraction was first carefully removed with a thin Pasteur pipette without disturbing the gradient. The remaining fractions were collected by elution through a small hole punctured at the bottom of the tube. The fractions were diluted 3–5 fold with Tris-Mg buffer, and centrifuged for one hour at 104,000 × *g*. The colorless supernatant liquids were discarded, the sides of the tubes were rinsed, and the pellets were resuspended in a small volume of Tris-Mg buffer.

Enzymatic assays: Photophosphorylating activity[14, 15] was measured by determining the disappearance of inorganic phosphate after 30 min of illumination

at 30°C ± 1°C under anaerobic conditions. The light intensity was approximately 1,000 ft.-c. at the surface of the reaction vessels. The reaction mixture contained: 20 μmoles of potassium phosphate buffer (pH 7.6), 1 μmole of ADP, 50 μmoles of mannose, 1 mg. of yeast hexokinase, 0.2 μmole of sodium succinate, 20 μmoles of $MgCl_2$, and 7–10 μmoles of Tris-HCl buffer (pH 7.6). The material to be assayed contained 1–5 mg. of protein, and 10–100 μg of bacteriochlorophyll. The total volume varied between 1 and 1.2 ml. In some experiments, methylphenazonium methosulfate (PMS) was added to the reaction system at a concentration of 0.2 mg per ml. In such cases, a piece of transparent cellophane, absorbing light below 500 mμ, was placed against the bottom of the reaction vessels to prevent destruction of the dye by light.[15] The reaction was performed in rectangular Warburg vessels in the Bronwill Warburg apparatus model UVL. The illuminated surface of the flasks measured approximately 10 cm,[2] and the reaction mixture accordingly had a depth of approximately 1 mm. Inorganic phosphate was measured by the method of Fiske and Subbarow[16] on an aliquot of the reaction mixture previously deproteinized by the addition of three volumes of 18 per cent trichloracetic acid. Two measures of specific photophosphorylative activity were used: micromoles of inorganic phosphate esterified per hour per milligram of chlorophyll; and micromoles of inorganic phosphate esterified per hour per milligram of protein. These values will be designed, respectively, as $Q_{Pi\ (chlorophyll)}$ and $Q_{Pi\ (protein)}$.

Succinic dehydrogenase activity was determined by manometric measurement of the oxygen uptake during 30 min at 30°C in the dark, in the presence of PMS.[17] The reaction mixture contained, in a final volume of 1.4 ml, 20 μmoles of sodium succinate, 100 μmoles of potassium phosphate (pH 7.4), 10 μmoles of $MgCl_2$, and 2 mg of PMS. The fractions assayed had a protein content of 0.5–1.5 mg. The reaction was started by tipping substrate and PMS from the side arm. The rate of oxygen uptake remained constant during the period of measurement. The very dilute preparations assayed consumed no oxygen in the absence of PMS, and the addition of KCN to block transport through the cytochrome chain could therefore be dispensed with. Phosphate was necessary for maximal activity. The specific activity of succinic dehydrogenase is expressed as μmoles of succinate oxidized per hour per mg protein, abbreviated as $Q_{succ.\ (protein)}$.

Purified pancreatic lipase was prepared from crude steapsin by the method of Glick and King.[18] Lipase digestion was carried out at 30°C for 2 hr, using 23 μg protein of purified lipase per ml of chromatophore suspension. The hexokinase and crude pancreatic lipase (steapsin) were products of the Nutritional Biochemicals Corporation.

Results.—Properties of the purified chromatophore fraction: Depending on the conditions of cultivation of the cells and the method of breakage employed, the purified chromatophore fraction represents from a third to a half of the chlorophyll-containing material recoverable from the sucrose gradient. Its specific chlorophyll content on a protein basis is always appreciably higher (by 10–30%) than that of the crude chromatophore preparation. The lower band, which we have termed the *heavy particle fraction*, always contains at least half and usually more of the chlorophyll-containing material recoverable from the gradient. Its specific chlorophyll content is in general slightly lower than that of the crude chromatophore preparation. The two enzymatic functions that we have examined—photophos-

phorylation and succinic dehydrogenase activity—occur in both purified chromatophores and heavy particles; specific activities are higher on a protein basis in the purified chromatophores than in crude chromatophores and heavy particles. Furthermore, the enrichment of the two functional properties in purified chromatophores parallels the enrichment of chlorophyll (Table 2). PMS increases photophosphorylative activity, but the relative increase is the same for each fraction.

TABLE 2

SPECIFIC ENZYMATIC ACTIVITIES OF CHROMATOPHORE FRACTIONS ISOLATED FROM PHOTOSYNTHETICALLY GROWN CELLS OF *Rhodospirillum rubrum*

	Crude chromatophores		Purified chromatophores		Heavy fraction	
	I*	II*	I	II	I	II
Cells grown at high light intensity (~2,000 ft.-c.)						
Specific chlorophyll: μg per mg protein	15.4	16.9	20.8	23.2	14.3	18.2
Photophosphorylation:						
$Q_{Pi\ (chlorophyll)}$ No PMS	547	485	738	645	283	241
$Q_{Pi\ (chlorophyll)}$ PMS	..	888	1,110	1,100	..	512
$Q_{Pi\ (protein)}$ No PMS	8.62	8.2	15.3	15	4.06	4.4
$Q_{Pi\ (protein)}$ PMS	..	15	25	25.7	..	9.12
Succinic dehydrogenase: $Q_{succ.\ (protein)}$	8.25	..	13	...	8.2	..
Cells grown at low light intensity (~200 ft.-c.)						
Specific chlorophyll: μg per mg protein	45.6	..	55.7	...	37.4	
Photophosphorylation:						
$Q_{Pi\ (chlorophyll)}$ No PMS	225	..	293	...	115	
$Q_{Pi\ (chlorophyll)}$ PMS	359	..	450	...	271	
$Q_{Pi\ (protein)}$ No PMS	10.4	..	16.3	...	4.32	
$Q_{Pi\ (protein)}$ PMS	16.5	..	25	..	10	
Succinic dehydrogenase: $Q_{succ.\ (protein)}$	7.4	..	12.6	...	7.3	

* I and * II represent two independent experiments.

Treatment with pancreatic lipase has a dramatic effect on the physical properties of the heavy particles. When a treated suspension of heavy particles is washed free of enzyme by centrifugation and then again subjected to a sucrose gradient centrifugation, all the chlorophyll-containing material can be recovered in particles that form a band in the gradient corresponding in position to that of purified chromatophores; however, the particles recoverable from the heavy fraction after lipase treatment have a higher specific chlorophyll content than the purified chromatophores isolated directly from the same crude chromatophore preparation. When the purified chromatophores are subjected to lipase treatment, their migration in the sucrose gradient does not change; however, their specific chlorophyll content increases substantially, to a value the same as that of the particles recoverable after lipase treatment of the heavy particle fraction. Data illustrating the effects of lipase treatment on purified chromatophores and on the heavy fraction prepared from cells grown at two different light intensities are shown in Table 3. It can be seen that the degree of chlorophyll enrichment achieved by lipase treatment of purified chromatophores is much greater for chromatophores prepared from cells grown at high light intensity, which have a low specific chlorophyll content, than

for the chromatophores prepared from cells grown at low light intensity. The particles obtained after lipase treatment retain the symmetrical infrared absorption band with a maximum at 885 mμ characteristic of the bacteriochlorophyll of *R. rubrum* in its native state; however, they are completely devoid of the capacity to photophosphorylate or to dehydrogenate succinate.

TABLE 3

EFFECT OF PANCREATIC LIPASE ON THE SPECIFIC CHLOROPHYLL CONTENT OF PURIFIED CHROMATOPHORES AND HEAVY PARTICULATE FRACTION OF *R. rubrum* GROWN PHOTOSYNTHETICALLY AT HIGH AND LOW LIGHT INTENSITY

	Specific chlorophyll: μg per mg protein	
	High light intensity (2,000 ft.-c.)	Low light intensity (200 ft.-c.)
Purified chromatophores	22.2	65.5
Heavy fraction	13	53
Lipase treated purified chromatophores	51	88.8
Lipase treated heavy fraction	54	87.5
Enrichment in specific chlorophyll after lipase treatment of purified chromatophores	2.3	1.35

In effect, therefore, the light fraction isolated by sucrose gradient centrifugation of crude chromatophore material is the most highly purified enzymatically functional chromatophore fraction that we have been able to isolate. A particulate fraction more highly purified in terms of pigment content is obtainable by lipase treatment; but such treatment abolishes enzymatic function. Since the primary aim of this work was to examine the relationship between the pigment content and the functional activity of the photosynthetic apparatus, the properties of the lipase-treated particles were not further investigated.

The relationship between the chlorophyll content of cells and chromatophores: Table 4 shows the specific chlorophyll content of crude bacterial extracts and purified

TABLE 4

SPECIFIC BACTERIOCHLOROPHYLL CONTENT OF WHOLE EXTRACTS AND PURIFIED CHROMATOPHORES PREPARED FROM *R. rubrum* GROWN PHOTOSYNTHETICALLY AT HIGH AND LOW LIGHT INTENSITY

	Specific bacteriochlorophyll content μg per mg protein		Percentage of total cell protein present in chromatophore material
	Crude extracts	Purified chromatophores	
Cells grown at high light intensity 2,000 ft.-c.	9.3	23.2	40
	10.2	24.9	41
	10.3	22.2	46.5
	11.7	24.4	48
Average value	10.4	23.7	45
Cells grown at low light intensity (200 ft.-c.)	26.7	49.5	54
	26.9	55.7	48
	35.8	65.5	55
	36.4	60.5	60
Average value	31.4	57.8	54

chromatophores prepared from four different batches of cells grown photosynthetically in malate medium under steady-state conditions of pigment synthesis at two different light intensities (*ca.* 200 and 2,000 ft.-c.). The specific chlorophyll content of crude extracts from cells grown at the lower light intensity is about three times as great as that of crude extracts from cells grown at the

higher light intensity. The purified chromatophores likewise show a very marked difference in specific chlorophyll content, paralleling that of the crude extracts. Hence it follows that the regulation of the chlorophyll content of whole cells in response to changes of light intensity is achieved primarily by a change in the chlorophyll content of the chromatophores, rather than by a change in the amount of chromatophore material per cell.

It is at present impossible to determine the chromatophore content of photosynthetic bacteria directly, since no method exists for the quantitative isolation of the photosynthetic apparatus in a physically homogeneous state. However, the fraction of cell protein represented by chromatophores can be derived from data on the specific chlorophyll content of whole extracts and of purified chromatophores. Calculated chromatophore contents, expressed in these terms, are shown in the last column of Table 4. The values suggest that the fraction of total cell protein represented by chromatophores is slightly greater in cells grown at low light intensity. The effect of light intensity on chromatophore content is, however, negligible in comparison with its effect on chlorophyll content.

The influence of chlorophyll content on the functional properties of chromatophores: Table 5 shows the specific activities of photophosphorylation and succinic dehydrogenase in purified chromatophores prepared from cells grown photosynthetically at different light intensities. The rate of photophosphorylation is presented both as a function of chlorophyll content—the measure of specific activity used by most workers[6]—and as a function of protein content. The latter values are relatively constant, and such variations as do occur are not systematically correlated with the light intensity at which the cells were grown. The former values vary almost sixfold, and are inversely related to the specific chlorophyll content of the chromatophores (Fig. 1). Since the rate of photophosphorylation was always measured at a saturating light intensity, it follows that this rate must have been limited not by the primary photochemical reaction (i.e., by the amount of chlorophyll present), but rather by one of the subsequent dark reactions. The specific activity of succinic dehydrogenase is remarkably constant, and does not differ significantly in chromatophores from cells grown at different light intensities (see also Table 2).

TABLE 5

ACTIVITIES OF PURIFIED CHROMATOPHORES EXTRACTED FROM *R. rubrum* GROWN PHOTOSYNTHETICALLY AT DIFFERENT LIGHT INTENSITIES

Approximate light intensity →	2,000 ft.-c. I	2,000 ft.-c. II	2,000 ft.-c. III	1,000 ft.-c. I	200 ft.-c. I	200 ft.-c. II	100 ft.-c. I
Specific chlorophyll: (μg per mg protein)	20.8	23.2	24.9	43	55.7	60.5	77.6
Photophosphorylation:							
$Q_{Pi\ (chlorophyll)}$							
No PMS	738	645	645	334	293	179	130
PMS	1,110	1,100	..	..	450	..	..
$Q_{Pi\ (protein)}$							
No PMS	15.3	15	14.4	14.7	16.3	10.8	10
PMS	25	25.7	..	..	25	..	..
Succinic dehydrogenase:							
$Q_{succ.\ (protein)}$	13.2	12.4	13	..	12.6	13.6	..

The formation of chlorophyll and chromatophores in cells initially free of pigment: Since air completely suppresses chlorophyll synthesis by *R. rubrum*, cells that have been grown for many generations in the dark under strictly aerobic conditions are devoid of chlorophyll. At very low oxygen tensions, such cells can initiate chlorophyll synthesis even in the absence of light.[19] Under these circumstances, growth is exceedingly slow. Nevertheless, chlorophyll synthesis is rapid, and after a few hours of incubation under conditions of oxygen limitation the cells attain a chlorophyll content comparable with that of cells grown anaerobically in the light (i.e., under conditions of obligatory photosynthesis). Preliminary experiments showed that the chromatophores isolated from such "semi-aerobic" cells, grown in the complete absence of light, could perform photophosphorylation. A detailed study of the synthesis of chlorophyll and the development of photophosphorylative capacity during semi-aerobic growth of initially depigmented cells was therefore undertaken.

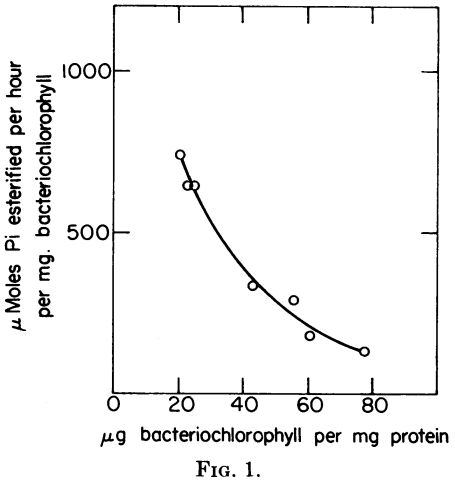


FIG. 1.

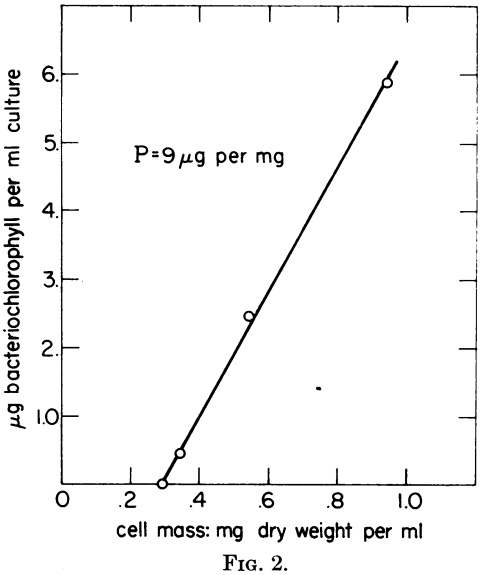


FIG. 2.

While still in the course of exponential growth under strictly aerobic conditions in the dark, several cultures of depigmented cells were pooled, and 750 ml of the pooled suspension was placed in each of several 1-liter Erlenmeyer flasks. These flasks were further incubated in the dark with gentle agitation. At appropriate intervals, the cells from a single flask were harvested for analysis. Oxygen is the limiting nutrient under these conditions, and the growth of the population is regulated by the rate of oxygen diffusion into the culture, and by the rate of its consumption. The kinetics of growth cannot, therefore, be precisely defined. Nevertheless, as shown in Figure 2, the increase in chlorophyll is strictly proportional to the increase in cell mass for several hours. In order to obtain a high differential rate of chlorophyll synthesis, the use of succinate as a carbon source is essential. Malate, although equivalent to succinate as a substrate for growth, supports little or no chlorophyll synthesis under semiaerobic conditions. With succinate as a carbon source, the differential rate of chlorophyll synthesis, P, under

semi-aerobic conditions in the dark approximates the differential rate of chlorophyll synthesis characteristic of cells growing photosynthetically under anaerobic conditions at a light intensity of 2,000 ft.-c. For example, in the experiment shown in Figure 2, the value of P is 9 μg per mg dry weight of cell material, as compared to a value of 7.5 μg per mg dry weight of cell material for cells growing photosynthetically at a light intensity of 2,000 ft.-c.

The specific chlorophyll contents of whole extracts and of the chromatophore fractions prepared from cells harvested between one and six hours after the initiation of pigment synthesis under semi-aerobic conditions in the dark are shown in Table 6. The parallelism between the values for extracts and for purified chromatophores is close. The fraction of cell protein represented by chromatophores, calculated from these data, ranged from 36.4 to 47.3%; it was, if anything, slightly higher in the early samples, but the significance of the variation is questionable. The average value for all samples was 41.5%, close to the figure of 45% calculated for cells grown anaerobically at a light intensity of 2,000 ft.-c.

TABLE 6

SPECIFIC BACTERIOCHLOROPHYLL CONTENT OF WHOLE EXTRACTS AND PURIFIED CHROMATOPHORE FRACTIONS FROM *R. rubrum* GROWN SEMI-AEROBICALLY IN THE DARK

Duration (hr) of semi-aerobic growth	Specific chlorophyll: μg per mg protein		Percentage of total cell protein present in chromatophore material
	Whole extract	Purified chromatophores	
1	1.55	3.28	47.3
1.5	2.55	5.51	46.3
2	2.68	7.37	36.4
4	9	22.6	40
6	11.8	31.5	37.5

The specific activities of photophosphorylation and succinic dehydrogenase in purified chromatophores from the same series of samples are shown in Table 7.

TABLE 7

ACTIVITIES OF PURIFIED CHROMATOPHORES PREPARED FROM INITIALLY DEPIGMENTED CELLS OF *R. rubrum* AFTER VARIOUS PERIODS OF GROWTH IN THE DARK UNDER SEMI-AEROBIC CONDITIONS

	Duration (hr) of semi-aerobic growth at time of harvesting and of chromatophore isolation						Average value for purified chromatophores from cells grown at a light intensity of 2,000 ft.-c.*
	1	1.5	2	4	6	6.5	
Specific chlorophyll of chromatophores: μg per mg protein	3.28	5.51	7.37	22.6	31.5	31	22.8
Photophosphorylation:							
$Q_{Pi\ (chlorophyll)}$							
No PMS	1,360	1,175	965	967	847	947	694
PMS	...	...	...	1,450	1,250	...	1,100
$Q_{Pi\ (protein)}$							
No PMS	4.45	6.5	7.1	21.8	26.6	29.3	15.3
PMS	...	...	...	32.8	39.4	...	25
Succinic dehydrogenase:							
$Q_{succ.\ (protein)}$	...	...	11.2	13.6	13.4	13.1	12.9

* From data in Table 3.

Calculated on a chlorophyll basis, the rate of photophosphorylation is highest in the early samples. These early values are far higher than any obtained for chromatophores of photosynthetically grown cells. As the chlorophyll content of the

chromatophores rises, the rate of photophosphorylation falls to a figure only slightly above that found for cells grown photosynthetically at a light intensity of 2,000 ft.-c. Taken as a whole, these figures suggest that even very soon after the initiation of pigment synthesis in the dark, cyclic photophosphorylation is not limited by the amount of chlorophyll in the photosynthetic apparatus. The rate of photophosphorylation calculated on a protein basis shows a quite different trend: there is a linear increase in specific activity as the chlorophyll content of the chromatophores rises (Fig. 3), and the final value obtained after 6 hr of semi-aerobic growth is much higher than any of the values found for photosynthetically grown cells. The specific succinic dehydrogenase activity of the chromatophores, on the other hand, is relatively constant, and of the same magnitude as that found in chromatophores from photosynthetically grown cells (*cf.* Table 5).

Two interpretations of the increase in the specific rate of photophosphorylation as measured on a protein basis are possible. This increase could imply that the chromatophore material formed early in the course of semi-aerobic growth has a low specific activity because it is deficient in one or more of the components required for the dark reactions of cyclic photophosphorylation, and that the specific activity subsequently rises as a result of increased formation of the specific limiting components. Alternatively, one could imagine that the photosynthetic apparatus is formed in association with particulate cell structures devoid themselves of photophosphorylative capacity, and that the purified chromatophores isolated soon after the initiation of pigment synthesis contain a large fraction of such photochemically inactive material. According to this interpretation, the steady rise in the rate of photophosphorylation measured on a protein basis would reflect a relative decrease in the amount of photochemically inactive material in the chromatophore fraction as chlorophyll synthesis proceeds. The constancy of specific succinic dehydrogenase activity in the successive chromatophore fractions would accordingly imply that this enzyme is present in both photosynthetically active and photosynthetically inactive components.

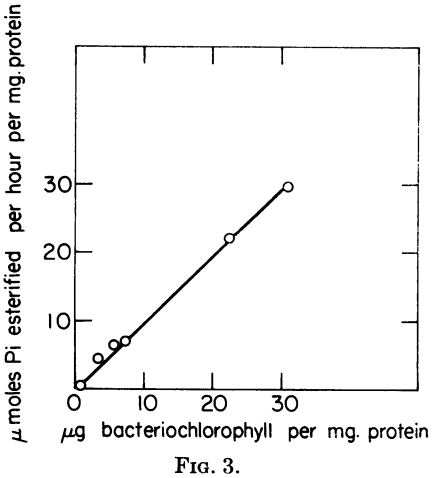


FIG. 3.

It is not possible to decide on the basis of the available information which of these two interpretations is correct. However, the relatively constant fraction of cell protein present in chromatophores throughout the course of semiaerobic pigment synthesis is most easily reconciled with the second interpretation: if the entire photosynthetic apparatus, as well as chlorophyll, were synthesized *de novo* from the start of semi-aerobic growth, the fraction of cell protein localized in the chromatophores should rise continuously.

Summary.—1. The purified chromatophores isolated from cells of *R. rubrum* grown anaerobically at high and low light intensities have markedly different chlorophyll contents, the differences being closely correlated with the differences in

chlorophyll content of the cells from which they were prepared. It is therefore evident that light-induced changes of cellular chlorophyll content reflect mainly changes in the amount of chlorophyll that is incorporated in the photosynthetic apparatus, rather than changes in the amount of chromatophore material in the cell.

2. In chromatophores prepared from cells growing photosynthetically under steady state conditions, the rate of photophosphorylation measured on the basis of chlorophyll content is inversely related to the amount of chlorophyll present in the chromatophores. Measured on the basis of protein content, however, the rate of phosphorylation is practically constant in chromatophores with very different chlorophyll contents. These facts indicate that the capacity for photophosphorylation is not limited by the amount of chlorophyll in the photosynthetic apparatus, but rather by some enzymatic component of the system.

3. When initially depigmented cells of *R. rubrum*, produced under strictly aerobic conditions of growth, are subjected to oxygen limitation in the dark, a rapid synthesis of chlorophyll ensues: the differential rate of chlorophyll synthesis is of the same order as that which occurs during anaerobic growth in the light.

4. The synthesis of chlorophyll under semi-aerobic conditions in the dark is accompanied by the formation of photochemically active chromatophores. The rate of photophosphorylation by such chromatophores as measured on the basis of protein content increases with the chlorophyll content of the cells, eventually attaining a value considerably higher than that characteristic of chromatophores prepared from cells grown under photosynthetic conditions.

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