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Effect of Light Intensity on the Formation of Intracytoplasmic Membrane in *Rhodospirillum rubrum*

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ABSTRACT

HOLT, STANLEY C. (University of California, Davis), AND ALLEN G. MARR. Effect of light intensity on the formation of intracytoplasmic membrane in *Rhodospirillum rubrum*. J. Bacteriol. **89**:1421-1429. 1965.—Cells of *Rhodospirillum rubrum* grown at low light intensity were found to contain much more internal membrane than cells grown at high light intensity. Highly purified membranes (chromatophores) from cells grown at low to moderate light intensity had a constant content of chlorophyll. Thus, the regulation of the chlorophyll content of the cell depends upon the formation of greater or lesser amounts of membrane which has a constant concentration of chlorophyll.

Photosynthetic bacteria are known to adapt to a decrease in light intensity by an increase in their content of chlorophyll. Cohen-Bazire, Sistrom, and Stanier (1957) found that in *Rhodopseudomonas spheroides* and *Rhodospirillum rubrum* the differential rate of chlorophyll synthesis is inversely related to the light intensity. After an increase in the light intensity, the bacterial population adjusts its pigment content by a transient suppression of the synthesis of pigment before the definitive differential rate is assumed. After a decrease in the light intensity, the differential rate of chlorophyll synthesis temporarily exceeds the definitive rate.

Sistrom (1962) demonstrated the obligatory coupling of pigment synthesis with protein synthesis in *R. spheroides* and proposed that the requirement for concomitant synthesis of protein reflects a requirement for the synthesis of membrane. This hypothesis excludes the incorporation of chlorophyll into existing membrane. Lascelles (1962) also presented evidence for the coupling of the synthesis of chlorophyll with the synthesis of protein in *R. spheroides*.

The evidence for the structural basis of the change in content of chlorophyll is equivocal. If the content of chlorophyll in the internal membranes is constant, the amount of membrane observed in thin sections should vary with light intensity. This variation has been observed (Cohen-Bazire and Kunisawa, 1963; Stanier, 1963). However, if the hypothesis is true, the content of chlorophyll in purified membranes should be independent of the light intensity; yet, a dependence has been reported (Cohen-Bazire and Kunisawa, 1960). This paper attempts to resolve this discrepancy.

R. rubrum was grown in a steady-state at each of several different light intensities. The relationship between light intensity and membrane content was established both by the examination of thin sections of cells emptied of cytoplasm by osmotic shock and by the determination of the specific chlorophyll content of membranes isolated from cells grown at various light intensities and purified by electrophoresis.

MATERIALS AND METHODS

Cultivation of bacteria. Rhodospirillum rubrum (strain S-1) was grown and harvested as previously described (Holt and Marr, 1965*a*). Cultures were growing exponentially at the time of harvest; as evidence of a steady-state, the differential rate of chlorophyll synthesis was found to be constant for at least two generations prior to harvest. The dry weight of cells was estimated by measuring the optical density (OD) in a 1-cm absorption cell with a Beckman DU spectrophotometer at 680 mμ, at which wavelength the photosynthetic pigments absorb minimally. The rear face of the absorption cell was 3.5 cm from the envelope of the blue-sensitive diode phototube, and the slit width of the monochromator was 0.04 cm. The OD over the range 0 to 0.3 is related linearly to dry weight; 0.1 OD is equivalent to 0.087 mg (dry weight) of cells per milliliter.

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Determination of chlorophyll. Chlorophyll was determined either by two wavelength spectrophotometry of suspensions of intact cells or by direct spectrophotometry after extraction. For the estimation of chlorophyll in suspensions of intact cells, the OD was measured at both 880 mμ (the absorption maximum for bacterio-chlorophyll in vivo) and 680 mμ. Since the OD of equivalent concentrations of pigmented and nonpigmented cells are equal at 680 mμ, the ratio (0.736) of the OD at 880 mμ to that at 680 mμ of suspensions of nonpigmented cells can be applied as a correction for the contribution of light scattering to the apparent absorbancy at 880 mμ. Thus, OD880-(OD 680) (0.736) is the absorbancy of the chlorophyll in pigmented cells. An absorbancy of 1.0^{-1} cm is equivalent to 9.5 mg of chlorophyll per milliliter.

Chlorophyll was determined after extraction by the procedure of Cohen-Bazire, Sistrom, and Stanier (1957).

Osmotic shock. The method used was that of Robrish and Marr (1962). A suspension of cells was mixed with an equal volume of 6 M glycerol. After 5 min, the mixture was drawn into a syringe and ejected into 10 volumes of mechanically stirred buffer [0.05 M tris(hydroxymethyl)aminomethane (Tris) HCl (*p*H 7.5) containing 0.001 M $MgCl_2$] at 4 C. The preparation was treated with 0.5 μg of deoxyribonuclease per ml for 20 min. The sample was then prefixed in 0.1% OsO_4.

Purification of intracellular membranes. Cells of *R. rubrum* were disrupted either by use of a French pressure cell or by osmotic shock. The isolated membranes were purified by density-gradient centrifugation and density-gradient electrophoresis as previously described (Holt and Marr, 1965*b*).

Electron microscopy. Specimens for electron microscopy were prepared and examined as previously described (Holt and Marr, 1965*a*). Samples were stained with lead hydroxide by the procedure of Millonig (1961).

RESULTS

Figure 1 shows the relationship between light intensity and both specific growth rate and specific chlorophyll content in the steady state. At light intensities above 400 ft-c the specific growth rate approaches a limiting value of 0.14 hr^{-1} and chlorophyll approaches a limiting value of 7.5 μg of chlorophyll per mg of protein. Below 100 ft-c, both specific growth rate and specific chlorophyll content are strong functions of the light intensity. At 1 ft-c, the lowest light intensity used in this study, the cells contain 41 μg of chlorophyll per milligram of protein, and the specific growth rate is 0.018 hr^{-1}.

Since it is difficult to establish the amount of intracytoplasmic membrane by electron micros-

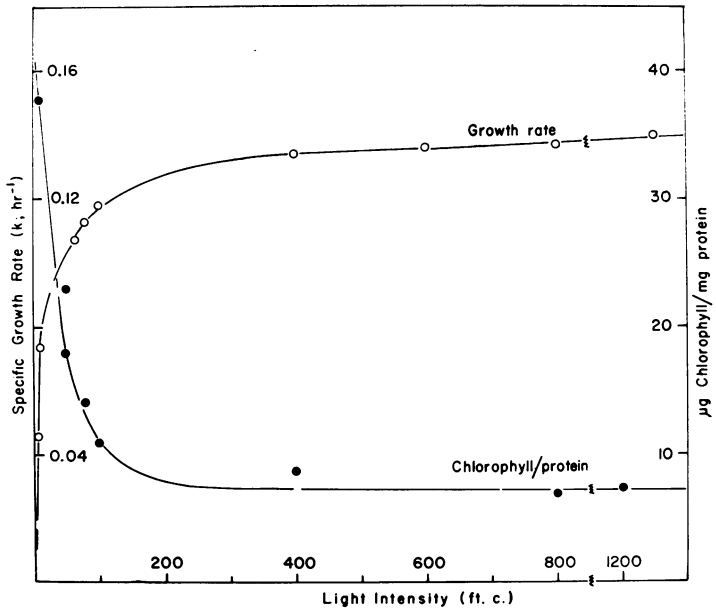


FIG. 1. *Specific growth rate and specific chlorophyll content of Rhodospirillum rubrum as functions of the light intensity. The specific growth rate, k, was computed as:*

$$k = \frac{2.303\ (\log_{10} x_2 - \log_{10} x_1)}{t_2 - t_1}$$

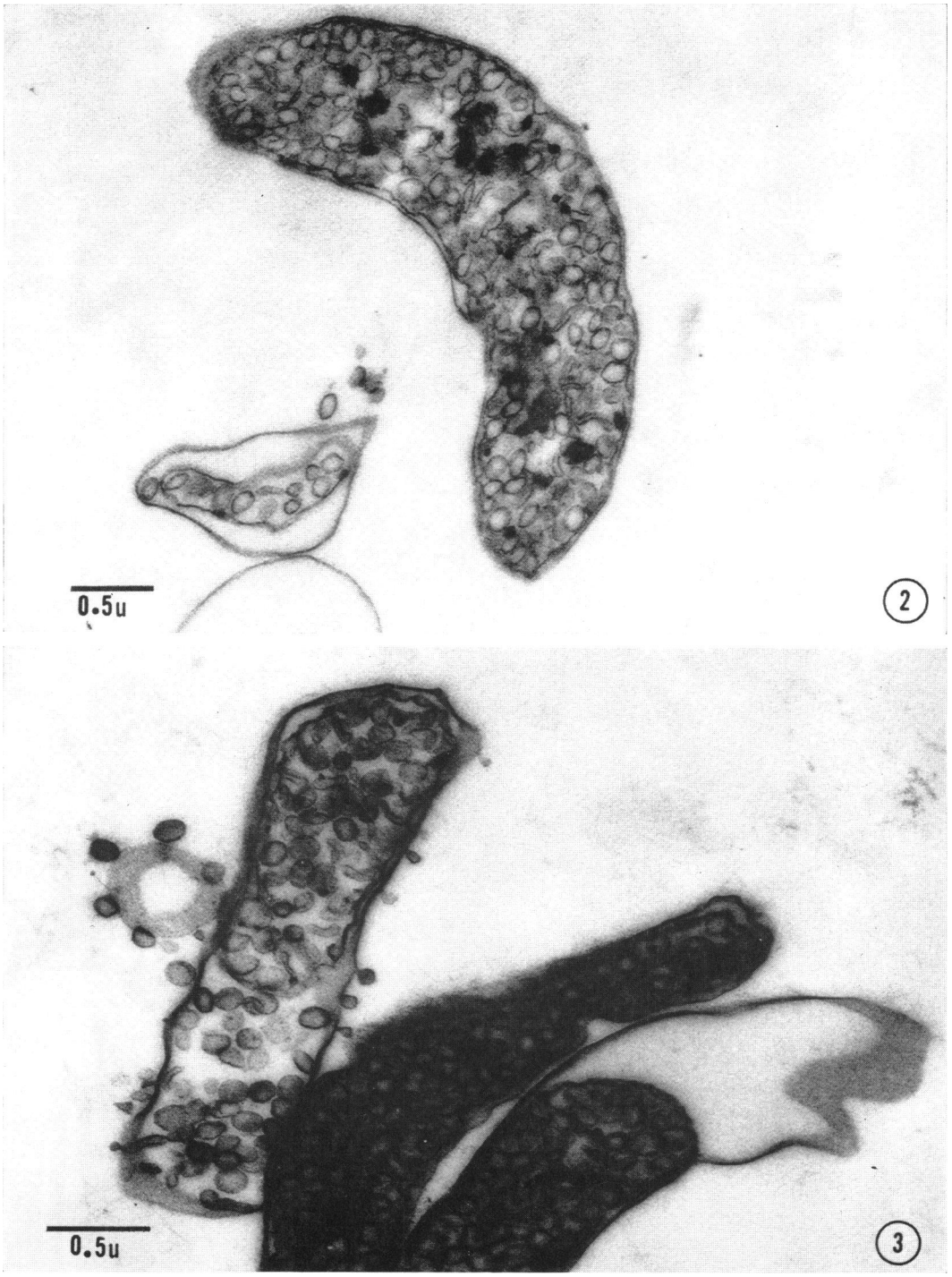


FIG. 2 and 3. *Rhodospirillum rubrum grown at 1 ft-c and osmotically shocked. Note the tubular appearance of the internal membranes. Main fixation, 12 hr; poststained with lead hydroxide.* × *32,000.*

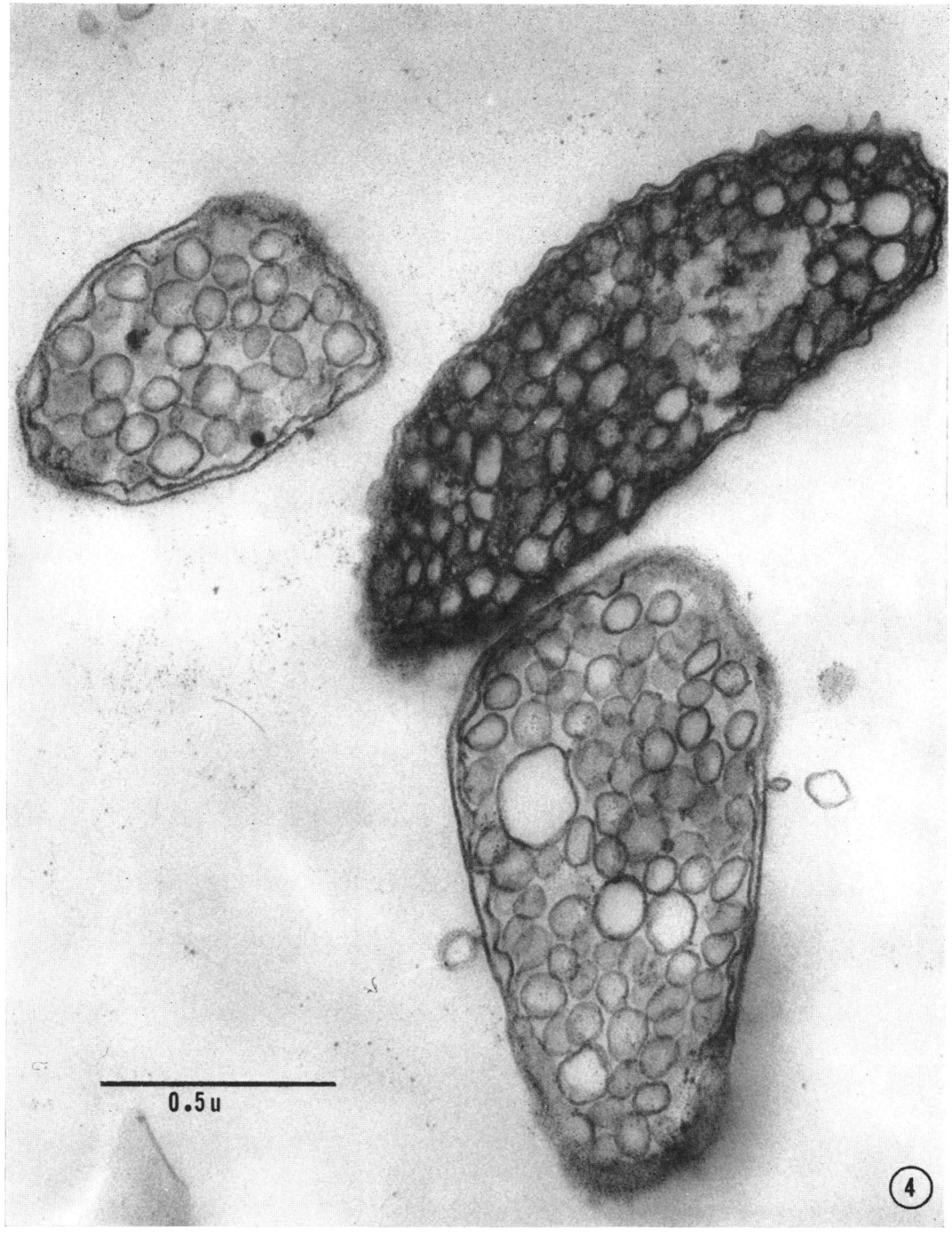
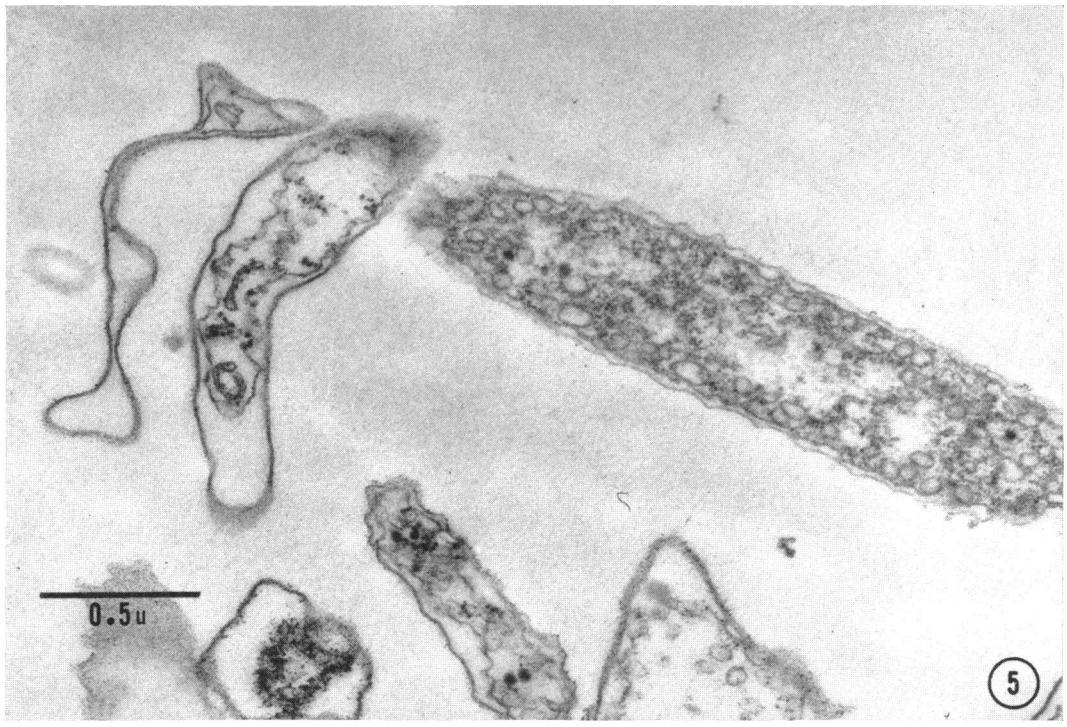


FIG. 4. *Sections of Rhodospirillum rubrum grown at 12 ft-c. Numerous interconnections between chromatophores are apparent in this micrograph. Main fixation, 12 hr; poststained with lead hydroxide.* × *76,500.*



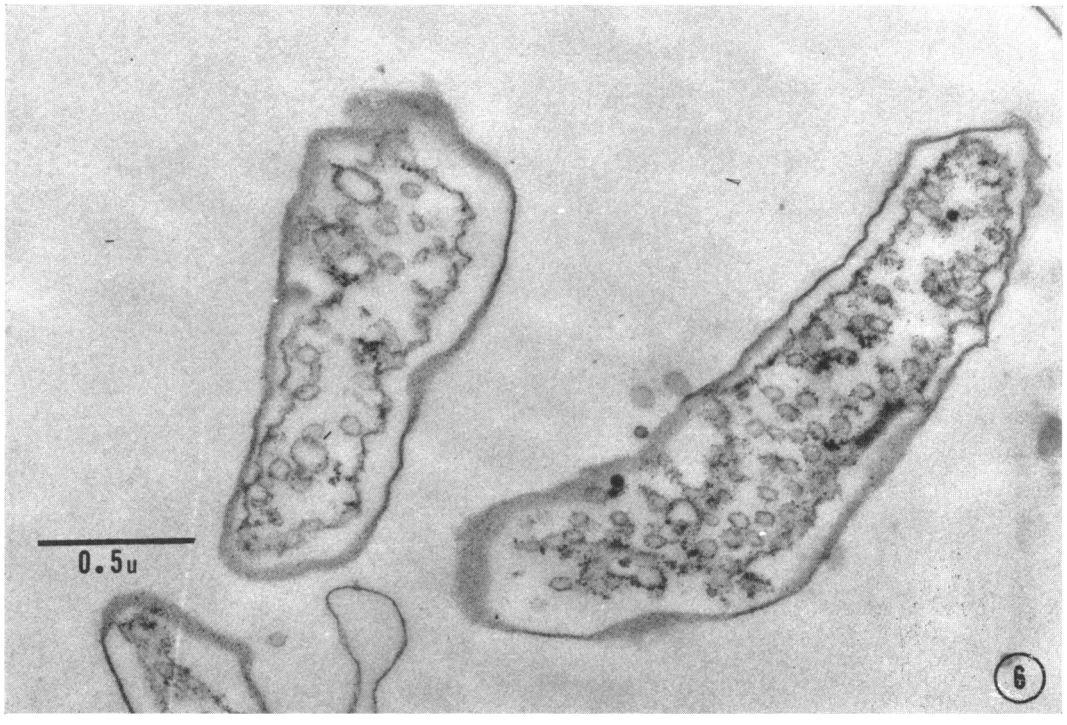
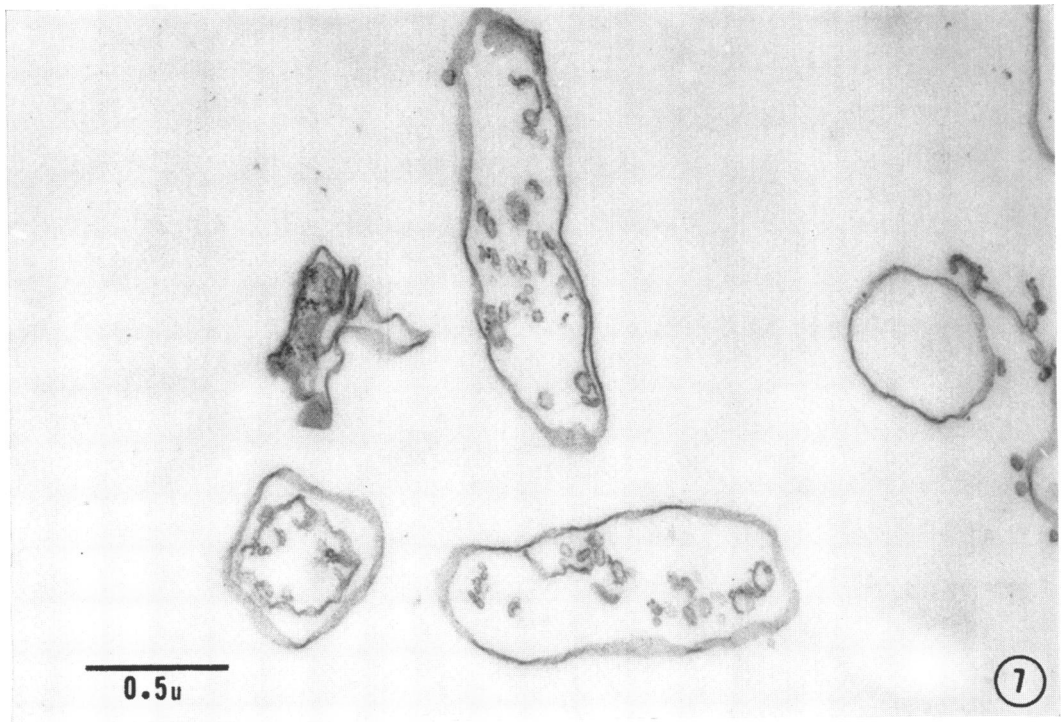


FIG. 5 and 6. *Sections of Rhodospirillum rubrum grown at 100 ft-c and osmotically shocked. The chromatophores are most numerous near the periphery of the cell. Interconnections of the chromatophores with the peripheral membrane are apparent. Main fixation, 12 hr; poststained with lead hydroxide.*



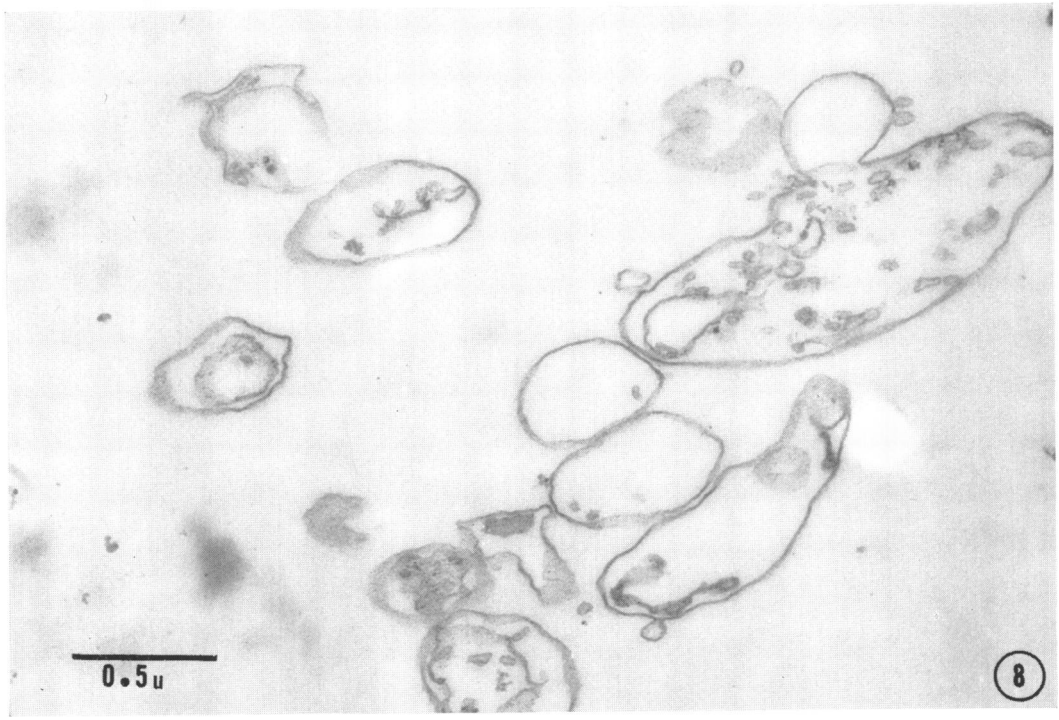


FIG. 7 and 8. *Rhodospirillum rubrum grown at 1,200 ft-c and osmotically shocked. Main fixation, 12 hr; poststained with lead hydroxide.* × *36,000.*

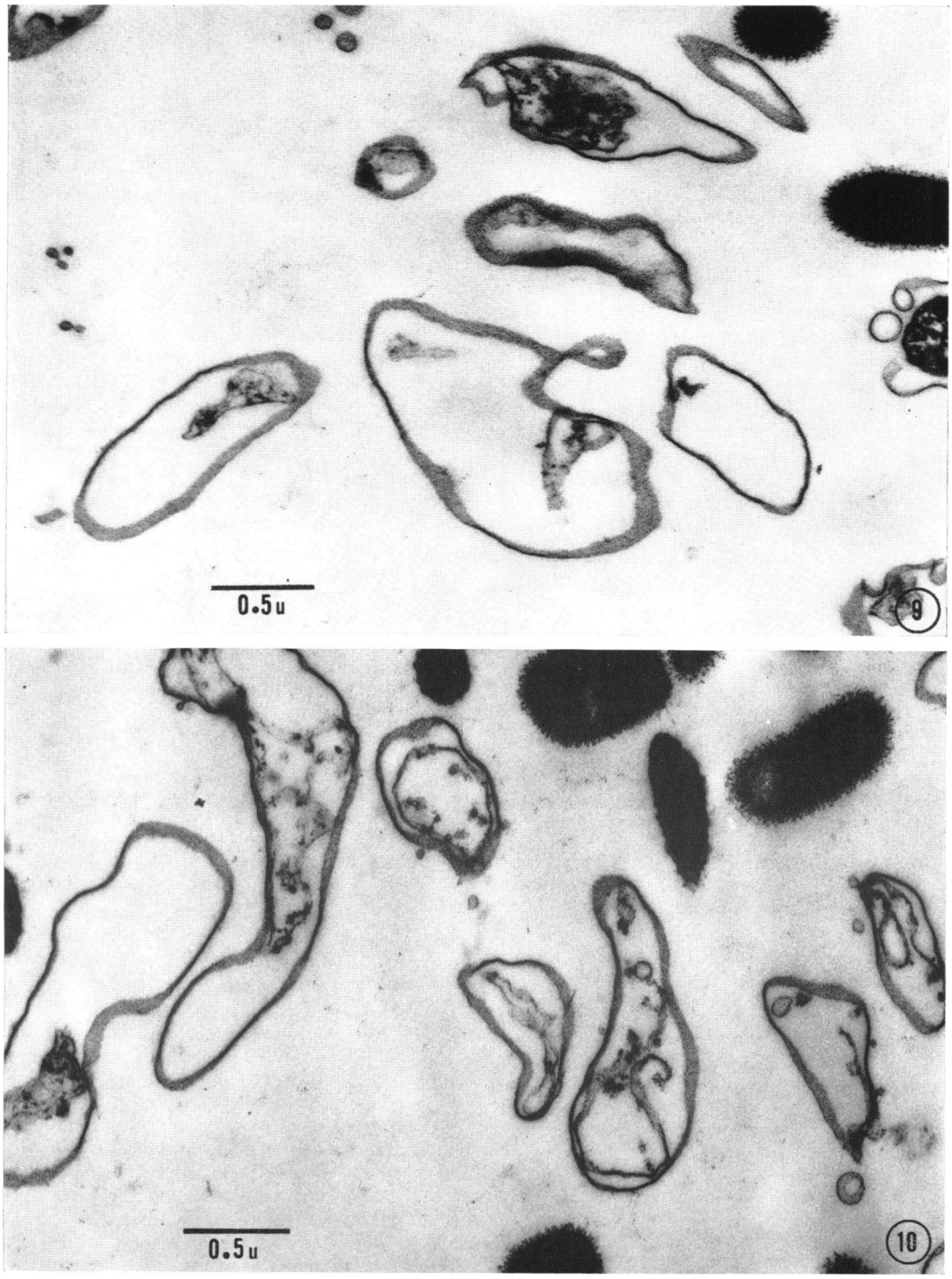


FIG. 9 and 10. *Rhodospirillum rubrum grown aerobically in the dark and osmotically shocked. Main fixation, 12 hr; poststained with lead hydroxide.* × *40,000.*

copy of sections of whole cells, *R. rubrum* was emptied of its cytoplasm by osmotic shock before fixation and sectioning.

The electron micrographs of osmotically shocked cells of *R. rubrum* establish that, as the light intensity is decreased, the content of internal membranes is increased. Figures 2, 3, and 4 show sections of cells grown at light intensities of 1 and 12 ft-c, respectively. The interior of the cells is filled with an extensive system of intracytoplasmic membranes. The internal membranes are far less abundant in cells grown at a light intensity of 100 ft-c (Fig. 5, 6). At 1,200 ft-c (Fig. 7, 8), the amount of membrane is significantly less than at 100 ft-c. Cells grown heterotrophically in the dark (Fig. 9, 10) contain few internal membranes.

The results of this survey of the structure of *R. rubrum* are in agreement with the results of Cohen-Bazire and Kunisawa (1963) and are in accord with the hypothesis that adaptation to the intensity of light results in a variation in the amount of membrane that is formed. However, the results do not establish that the concentration of chlorophyll in the internal membrane is constant. This was established by the analysis of purified membranes (chromatophores) from cells grown at four different light intensities. The cells were disrupted either by osmotic shock or by treatment in the French pressure cell, and the membranes were purified by two centrifugations in a density gradient followed by electrophoresis (Holt and Marr, 1965*b*).

TABLE 1. *Chlorophyll content of purified membranes isolated from Rhodospirillum rubrum grown at different light intensities*

Sample	Light intensity (ft-c)			
	1	100	2,400	6,000
Intact cells.....	28.2*	7.20	3.21	2.36
Total extract.....	41.6†	11.6	6.17	4.12
Purified‡ membranes.....	86.4†	86.2	70.0	44.0

* Amounts for intact cells expressed as micrograms of chlorophyll per milligram (dry weight).

† Amounts for total extract and purified membranes expressed as micrograms of chlorophyll per milligram of protein.

‡ Purified by two successive centrifugations in sucrose density gradients followed by electrophoresis.

TABLE 2. *Comparison of the chlorophyll content of membranes from Rhodospirillum rubrum*

Prepn	Low light intensity (100 ft-c)	High light intensity (2,000 to 2,400 ft-c)
Membranes isolated by Cohen-Bazire and Kunisawa (1960).....	77.6*	23*
Crude membranes.....	64.8	30.3
Purified membranes†.....	86.2	70.0

* Results are given as micrograms of chlorophyll per milligram of protein.

† Purified by two successive centrifugations in a sucrose gradient followed by electrophoresis.

The chlorophyll content of the purified membranes grown at different light intensities is shown in Table 1. Although the specific chlorophyll content of the total extract varies sevenfold, the specific chlorophyll content of the purified membranes is almost independent of the light intensity up to 2,400 ft-c. At 6,000 ft-c, the specific chlorophyll content of the purified membranes decreases significantly.

Thus, at low and moderate light intensities the adaptation which results from a change in the light intensity results in the formation of more or less internal membrane which has a constant content of chlorophyll. At very high light intensities, the specific content of chlorophyll in the purified membrane decreases.

DISCUSSION

The effect of light intensity on the synthesis of chlorophyll and intracytoplasmic membrane in *R. rubrum* was investigated previously by Cohen-Bazire and Kunisawa (1963). In cells growing anaerobically in the light, the differential rate of chlorophyll synthesis was found to be inversely related to the light intensity. Cells growing at low light intensity were found to synthesize more chlorophyll than cells growing phototrophically at high light intensity or chemotrophically in the dark.

The micrographs presented by Cohen-Bazire and Kunisawa (1963) and Stanier (1963) establish a variation in the amount of membrane with a change in the light intensity. In sections of whole cells of *R. rubrum* growing at high light intensity, the internal membranes (chromatophores) are restricted to a peripheral location. At low light intensities, the membranes are more abundant and intrude into the cytoplasm.

It is difficult to assess the amount of internal membrane by electron microscopy of sections of whole cells, because ribosomes obscure the membranes. Since the ribosome content varies with growth rate (Ecker and Schaechter, 1963), and

since *R. rubrum* has a very low growth rate at low light intensities, an apparent change in the amount of membrane might result from a change in the concentration of ribosomes. In an attempt to avoid this difficulty, the cells of *R. rubrum* were emptied of cytoplasm by osmotic lysis. The relative amount of internal membranes in the emptied cells confirms the conclusion that the amount of internal membrane is much greater if cells are grown at low rather than at moderate or high light intensity.

In support of the idea that an increased content of chlorophyll results in a corresponding increase in the amount of internal membrane is the constancy of the thickness of the membrane. The thickness is approximately 70 to 80 A and is independent of the intensity of light. A large change in the concentration of photopigments could not be accommodated without an increase in the thickness of the membrane.

If the amount of internal membrane increases in proportion to the increase in the content of chlorophyll, the concentration of chlorophyll in the membrane should be constant. Cohen-Bazire and Kunisawa (1963) found that the specific chlorophyll content of the isolated membranes varied with the light intensity at which the cells were grown. The chlorophyll content of the purified membranes was found to vary directly with the chlorophyll content of the cells from which the membranes were isolated. In contradiction to these results, we found that the specific chlorophyll content of the purified membranes is almost independent of the specific chlorophyll content of cells grown at low to moderate light intensities. Table 2 compares both sets of data. The variation in chlorophyll content with the light intensity of growth is almost insignificant after purification by electrophoresis. Presumably, the previously observed variability of chlorophyll content results from a variable contamination by cytoplasmic components which are removed by electrophoresis.

We conclude that the regulation of the chlorophyll content in response to changes in light intensity is not achieved by a change in the specific chlorophyll content of the internal membranes, but by a change in the quantity of membranes of constant composition.

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