PIGMENT CARRYING STRUCTURES OF THE PHOTOSYNTHETIC NON-SULPHUR PURPLE BACTERIUM RHODOPSEUDOMONAS SPHEROIDES

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As observed under the electron microscope (6), aqueous extracts of photosynthetic bacteria contain granula-like structures; most probably, these organelles are built up of lamellae. Pardee, Schachman and Stanier (5) showed that the colored sediment obtained by centrifugation of aqueous extracts of *Rhodospirillum rubrum* consisted of the above-mentioned granula-like structures. Niklowitz and Drews (4) prepared ultra-thin sections of *Rhodospirillum rubrum*. Electron microscopical examination revealed that this photosynthetic bacterium contains lamellated structures. This was confirmed by Elbers, Minnaert and Thomas (2). On the other hand, Vatter and Wolfe (9) observed spherical organelles packed throughout the bacterial cell. These organelles were considered to be chromatophores. Vatter and Wolfe (9, 10) stated that light is essential for the formation of these chromatophores. The latter authors studied a number of photosynthetic bacteria, among which was *Rhodopseudomonas spheroides*.

Thus, the results seem to be conflicting. A private discussion, however, revealed that the age of the bacteria may be responsible for this discrepancy. Vatter and Wolfe used bacteria only a few days old, whereas Elbers et al studied 7- to 10-day-old cultures which were still in a photosynthetically active state.

Goedheer (3) studied polarization of bacteriochlorophyll fluorescence in *Rhodospirillum rubrum* and Chromatium. Since, be it only to a relatively small degree, polarization was found to occur, it was concluded that the pigment molecules are preferentially oriented. This conclusion favors the conception of the occurrence of flat lamellae rather than that of vesicles. In any case, if the bacteriochlorophyll molecules were attached to the surface of vesicles, these structures should be flattened in a preferential plane. It would be interesting to study polarization of bacteriochlorophyll in both young and aged cultures.

Though it seems likely, evidence that bacteriochlorophyll is connected with lamellar structures in some way or another is so far lacking. Working with *Hibiscus rosa sinensis*, Thomas, Post and Vertregt (8) obtained the indication that, in the living cell, the occurrence of chlorophyll is restricted to the grana lamellae. They used the Molisch reaction, i.e. silver nitrate reduction in the presence of excited chlorophyll, as a tool for localization of this pigment. However, because of the small dimensions of the bacterial photosynthetic apparatus, the silver nitrate method cannot be expected to yield reliable results. For this reason, attempts were made to localize the photosynthetic pigments within the bacterial cell by studying ultra-thin sections of the colored fraction of *Rhodopseudomonas* homogenates under the electron microscope. The present paper shows the results of this attempt.

**Materials and Methods**

*Rhodopseudomonas spheroides* cells, grown in a 1% peptone and 0.5% NaCl medium, were collected from about 10-day-old cultures by centrifugation. They were resuspended in 0.1 M TRIS buffer of pH 8.0. This suspension was sonicated at 7 Kc in a magnewtrestriction oscillator for 30 minutes. During disintegration, the suspension was cooled with tap water. The colored material was separated by differential centrifugation and repeatedly washed in a Spinco ultracentrifuge with the buffer solution. Finally, the clear and colorless supernate was decanted and the sediment, which seemed to consist entirely of colored matter, was used for examination. A detailed description of this procedure will be published by Bril (1).

The sediment was fixed with 1% osmium tetroxide solution, phosphate-buffered at pH 7.2, and embedded in methylmethacrylate as described by Elbers et al (2). Preparation and sectioning were carried out by the Technical Physical Service T.N.O. and T.H., Delft, who also made the electron micrographs. The thickness of the sections, prepared with a Philips ultramicrotome, was about 200 Å.

**Results**

Figure 1 shows an electron micrograph of a section through sonicated and purified colored matter from *Rhodopseudomonas spheroides*. Evidently, this disintegrated fraction consists almost entirely of lamellar material. As a rule, the cross-sectioned lamellae show up as curved lines. Since one never is certain whether a lamella was oriented in a plane perpendicular to the plane of cutting, it is impossible, to determine the lamellar thickness without doubt. It can only be stated that, probably, the thickness of the lamellae is about 30 Å.

As far as the lamellae occurred in a plane parallel to the plane of cutting, or under a slight angle to it, they can be observed as homogenous half-tones. Globular cytoplasmatic matter seems to be nearly, if not totally, absent in the preparation. Since the occurrence of colored material in the homogenate is re-

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stricted to the fraction under consideration, it may be concluded that, most probably, the photosynthetic pigments occur in, or at the surface of, lamellae in the bacterium studied.

**Discussion**

Instead of grinding, sonic treatment was chosen as a means to disintegrate the bacteria. This was done in order to obtain a high degree of separation of the structural elements from each other, and, thus, to enable thorough washing of the pigment containing fraction. However, this method of disintegration is likely to cause a more or less serious distortion of the material studied. As a consequence, it is impossible to decide whether the curling of the lamellae, as shown in figure 1, should be considered an artifact or representation of the original shape. Moreover, distortion of the isolated lamellae might also be caused by the fixation technique which was used. For these reasons, the above result is not definitive with regard to establishment of the shape of the photosynthetic organelle in the living cell.

The data under consideration indicate that, in the photosynthetic bacterium *Rhodopseudomonas spheroides*, the pigments are attached to lamellae. Such a situation probably occurs in higher plants (8). Thus, the present investigation suggests that the structural units of the photosynthetic apparatuses of both the bacterium and the higher plants are similarly constituted. This suggestion favors the conception (7) that arrangement of chlorophyllous pigments on lamellar structures is essential for the functioning of these pigments in photosynthesis.

**Summary**

1. Ultra-thin sections of the pigment-containing fraction of sonically disintegrated cultures of *Rhodopseudomonas spheroides* were studied under the electron microscope.

2. This fraction was found to consist of lamellar material of a thickness of, probably, about 30 Å.

![Fig. 1. Electron micrograph of a section through the pigment-carrying fraction from a homogenate of *Rhodopseudomonas spheroides*.](image-url)
3. It was concluded that the photosynthetic pigments of the studied bacterium are likely to be attached to lamellae.

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**LITERATURE CITED**

1. Bril, C. (In preparation.)

**FORMATION AND BLEACHING OF CHLOROPHYLL IN ALBINO CORN SEEDLINGS**

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In 1951 Koski and Smith (5) found that albino seedlings of corn formed protochlorophyll and chlorophyll equally as well as normal plants. These albinos lacked the ability to accumulate chlorophyll, however, because the chlorophyll was bleached by continued illumination. Several factors were suspected of causing the instability of the chlorophyll, and this paper presents an examination of these factors.

Granick (3) observed that a Chlorella mutant which formed only photochlorophyllide (i.e., phytlyl-free protochlorophyll) bleached in the light. He suggested that the loss of pigment was caused by the lack of the phytly group. Loeffler (6, 11) and Wolff and Price (15) discovered independently that normal seedlings grown in the dark formed both protochlorophyll and protochlorophyllide, which they transformed to chlorophyll a and chlorophyllide a in the light and subsequently esterified the chlorophyllide a to chlorophyll a. If, in accordance with Granick's suggestion, the albino plants contained protochlorophyllide and were unable to completely esterify (phytlylate) the chlorophyllide formed therefrom, this might be a cause of albinism. For this reason a number of albino plants were examined for their esterifying ability.

Another suspected cause of bleaching was that albino plants did not stabilize the chlorophyll after it was formed. Shibata (8) discovered that in normal plants the chlorophyll first formed from transforma-

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