Worthington Biochemical Corporation (1963), Freehold, N. J.
Wright, B. E. (1964), *in* The Protozoa, Vol. III, Hutner, S. H., ed., New York, Academic, p. 341.
Wright, B. E., Brühmüller, M., and Ward, C. (1964), *Develop. Biol. 9*, 287.
Wright, B. E., and Ward, C. (1965), *Bacteriological Proc.*, p. 35.

Nature of the Insoluble Pigmented Structures (Chromatophores) in Extracts and Lysates of *Rhodopseudomonas spheroides**

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ABSTRACT: When extracts of anaerobically grown *Rhodopseudomonas spheroides*, made with a French press, were centrifuged in a linear gradient of cesium chloride the great majority of the pigment was found in a single narrow band. Four other protein-containing bands could be identified. Extracts of aerobic cells contained the same bands apart from the pigmented one. Examination of anaerobic extracts by moving boundary sedimentation showed two types of pigmented particle whose $s_{20,w}$ values were 160 S and 55 S and whose densities were 1.17–1.18. The larger particle contained 85% or more of the pigment. An estimate of the polydispersity of the 160 S particles showed that the standard deviation of the distribution of their diameters was equal to 9–12% of its mean. From these results and others [K. D. Gibson, 1965c, *J. Bacteriol.* (in press)] the particles were calculated to consist of about 50% water. Anaerobic cells were converted to spheroplasts and lysed by various methods. Most of the pigment remained attached to large fragments, but a small amount was released in the form of 160 S particles. Application of shearing forces liberated more pigmented particles. The results were consistent with the view that the pigmented structure is particulate *in vivo*.

Schachman *et al.* (1952) found that extracts of anaerobically grown *Rhodospirillum rubrum*, when observed in the analytical ultracentrifuge, contained particles whose $s_{20,w}$ value was 155 S, with which nearly all the photosynthetic pigment was associated. They named these particles chromatophores. The remainder of the pigment was apparently contained in smaller particles whose $s_{20,w}$ value was 55 S. The two fractions seemed to be fairly homogeneous, and it was possible to isolate some material corresponding to the larger particles by differential centrifugation. In the next few years a number of reports appeared describing the isolation of pigmented particles from extracts of *Chromatium* (Newton and Newton, 1957; Bergeron, 1958), *Chlorobium thiosulfatophilum* (Bergeron and Fuller, 1961), *R. rubrum* (Frenkel and Hickman, 1959; Cohen-Bazire and Kunisawa, 1960), and *Rhodopseudomonas spheroides* (Bull and Lascelles, 1963; Worden and Sistrom, 1964). In these studies the cells were ruptured by sonication, grinding with abrasives, or by passage through the Hughes press or French press. The isolated particles were for the most part active in catalyzing photophosphorylation (Frenkel, 1954) and certain photooxidations and reductions (Vernon and Ash, 1959), and their composition was fairly similar in all these species. The structures in the cell from which the pigmented particles arose were identified in electron micrographs of thin sections of whole cells of *Chromatium*, *R. rubrum*, and *Rps. spheroides* as rather uniform electron-lucent vesicles bounded by electron-dense membranes, which are distributed in clumps throughout the interior of the cell, almost filling it in extreme cases (Vatter and Wolfe, 1958; Bergeron, 1958; Hickman and Frenkel, 1959; Cohen-Bazire and Kunisawa, 1963; Cohen-Bazire, 1963).

However, there were some observations which suggest that the pigmented structure is not particulate *in vivo*, but consists of a reticulum which becomes comminuted during mechanical rupture of the cell in a manner analogous to the conversion of the reticular endothelium to microsomes. The pigment did not seem to be in a single subcellular fraction but could be found in at least two types of particle (Newton and Newton, 1957; Cohen-Bazire and Kunisawa, 1960). These were named "heavy" and "light" chromatophores. It appeared that the "heavy" fraction consisted of "light" particles attached to other components of the cell, indicating that *in vivo* the particles formed part of a larger structure. More recently it was observed that the membrane-bound vesicles which are seen in electron micro-

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graphs of thin sections of cells are not all separate, but that a small number of them appear to merge with the cytoplasmic membrane to form a structure resembling a bud (Cohen-Bazire and Kunisawa, 1963; Boatman, 1964). Also when extracts of *R. rubrum* were obtained by lysis of spheroplasts produced with lysozyme and EDTA (Karunairatnam *et al.*, 1958) or by treatment with lysozyme and polymyxin B (Tuttle and Gest, 1959) the pigment was all released in a form which sedimented readily in low centrifugal fields while retaining full catalytic activity. Small pigmented particles could be released from these fractions by sonication or by addition of detergents. On the basis of these observations it was suggested that the pigment is actually present in the cell in a membranous structure which originates from the cytoplasmic membrane and perhaps remains attached to it, and that this structure becomes comminuted into small particles when subjected to mechanical procedures of cell rupture.

The purpose of this and the accompanying paper (Gibson, 1965a) is to present evidence indicating that at least in the case of *Rps. spheroides* the original view of Schachman *et al.* (1952) is nearer the truth than is the view of Gest and Cohen-Bazire and their collaborators. In this paper the distribution of pigment in extracts of this organism is investigated by zone centrifugation on gradients of CsCl and by the moving boundary sedimentation technique of Trautman and Breese (1959), and it is shown that nearly all the pigment is present in a single, rather homogeneous particulate fraction. The isolation of these particles and some of their properties are described in the next paper (Gibson, 1965a).

Materials and Methods

Growth of Organisms and Preparation of Extracts. The organism used in this study was a streptomycin-resistant mutant of *Rps. spheroides* strain S, obtained by seeding a plate of solid (1% agar) medium containing streptomycin sulfate (0.5 mg/ml) with about 10^8 cells/ml and incubating it under light for 5 days. Cultures were grown either semiaerobically in the light or aerobically in the dark as described previously (Gibson *et al.*, 1962), except that the medium employed for all cultures was the one described by Cohen-Bazire *et al.* (1957) with the Casamino acids omitted and 0.1% sodium glutamate included in their place. At the light intensities used routinely in this laboratory (about 150–200 foot candles) light-grown cultures had bacteriochlorophyll contents in the range 15–25 μmmoles/mg dry weight.

Cultures were harvested by centrifugation, washed once with water, and resuspended in Tris buffer (0.002 to 0.01 M), pH 7.5, at concentrations of 25–100 mg/ml. They were then passed through a French press (French and Milner, 1955) at 5°, operated under a pressure of 8–10 tons/in.^{2}, at a rate of approximately 5–10 ml/min. The extracts were cooled in ice as they emerged from the press. A single passage through the apparatus in use in this laboratory normally ruptured 80–90% of the cells originally present as judged by microscopic examination. In certain experiments extracts were prepared by lysis of the cell wall by one of three methods involving the use of lysozyme. The first method was to convert the organisms to spheroplasts with lysozyme and EDTA in the presence of 10% sucrose, in the manner described for *R. rubrum* (Karunairatnam *et al.*, 1958), and to lyse the washed spheroplasts by suspending them in a small volume of 0.001 M Tris buffer. The second method employed lysozyme and polymyxin B. Again the procedure was the same as that employed for *R. rubrum* (Tuttle and Gest, 1959), except that the concentration of lysozyme was increased to 500 μg/ml and that of polymyxin B to 100 μg/ml. The third method made use of the conversion of Gram-negative bacteria to spheroplasts which occurs in the presence of penicillin and its derivatives. α-Aminobenzylpenicillin (ampicillin) was added at a concentration of 30 μg/ml to a culture of organisms (1 mg/ml) growing in the light, and incubation was continued for a further 12 hr. The organisms were harvested, washed, and suspended in 0.01 M Tris buffer, pH 7.5, at a concentration of 10 mg/ml. Lysozyme (500 μg/ml) was added and the mixture incubated at 37° for 2 hr.

ZONE CENTRIFUGATION. This was carried out in linear gradients of CsCl in the SW39L rotor of the Spinco Model L ultracentrifuge. The gradients were established by mixing solutions containing 0.60 M CsCl and 1.66 M CsCl in 0.01 M Tris buffer, pH 7.5 (2.4 ml of each), in a standard apparatus (Britten and Roberts, 1960). Such gradients are stable overnight. The sample (up to 0.4 ml) to be analyzed was layered on top of the gradient and the tube was centrifuged at 39,000 rpm for 100 min plus the time required for acceleration and deceleration. The contents of the tube were withdrawn through a capillary with an inverted tip inserted in such a way as to collect from the lowest point of the cylindrical part of the tube. The procedure appeared to cause about the same amount of distortion as does the more usual method of collection through a hole in the bottom of the tube; however, it did not disturb the pellet, which could be collected separately. The effluent from the capillary was divided into 35–40 fractions by means of a drop-counting device, and the various particulate fractions of the cell were located by determining the optical densities of the fractions at 260, 280, and 850 mμ (the latter wavelength is an absorption maximum for bacteriochlorophyll *in vivo*).

MOVING BOUNDARY SEDIMENTATION. This was performed in the SW39L rotor of the Spinco Model L, using linear gradients of KCl to discourage convection. The preparation to be analyzed was divided into two portions of 2.5 ml each. One portion was placed directly in the reservoir of the gradient device, and solid KCl (100 mg) was dissolved in the other portion, after which it was placed in the mixing chamber. In this way a linear gradient of KCl was established in which the concentration of KCl at the bottom of the tube was about 4% (w/w) greater than that at the top. The tubes were then centrifuged in the SW39L rotor of the Spinco, care being taken to avoid rapid acceleration or deceleration.

At the end of each run the distribution of pigment in the tube was determined by running its contents through

an Autoanalyser (Technicon) and following the absorption due to carotenoid at 440 mμ. Two methods of sampling were used. In the first method a capillary with a bent tip was inserted near the bottom of the tube and the material was withdrawn through it at a rate of 0.16 ml/min. In the second method 30% sucrose was pumped into the bottom of the tube by means of the proportioning pump and the contents were withdrawn at the same rate through another capillary at the meniscus. The material withdrawn from the tubes by either method was mixed with 12.5 volumes of 10^{-3} M Tris buffer, pH 7.5, in the proportioning pump and passed directly into the colorimeter.

Calculation of Sedimentation Coefficients. The position of a boundary was located by the first method of Gibson (1965b). This method was preferred to the simpler alternative method because the results indicated that there were two types of particle whose initial concentrations could not be determined individually, and also because the concentrations at points near the bottom of the tube could be determined with a high degree of accuracy because of the techniques employed for analyzing the contents of the tubes. To calculate the sedimentation coefficients of the two types of particle individually it was necessary to assume that above a certain point in the tube all the pigment was present in the smaller particle while below this point it was all present in the larger particle. The point of division was taken to be at the point of inflexion of the experimental curve (X in Figure 3). In fact there is some latitude allowed in the choice of the point of division because errors introduced in this way tend to cancel out during the calculation of the position of the boundary, as can be seen from a consideration of the appropriate equations given by Gibson (1965b). It was assumed that the concentration of the smaller particles was constant below the point of division. To calculate the position of the lower boundary due to the larger particle, it was found necessary to proceed only to the second approximation as the first correction obtained was always less than 0.25 cm. The second correction to the boundary was less than 0.03 cm and was ignored as the accuracy of the method did not seem to justify its use. The mean sedimentation coefficients were calculated from the positions of the boundaries by the formula

$$s = \frac{1}{\omega^2 t} \ln \frac{x_t}{x_0} \qquad (1)$$

where x_0 is the initial coordinate (distance from the axis of centrifugation) of the boundary and x_t is its coordinate at time t while ω is the angular velocity. One-third of the times for acceleration and deceleration were included in the total time t of sedimentation.

The sedimentation coefficients determined in this way were used to determine the diameter d and density ρ_0 of the equivalent hydrodynamic sphere from the formula

$$\eta s = \frac{d^2}{18q} (\rho_0 - \rho) \qquad (2)$$

where ρ and η are the density and viscosity of the solution and q is the frictional ratio of the particle (usually written f/f_0). The quantities d^2/q and ρ_0 were obtained from the slope and intercept of the lines obtained by plotting ηs against ρ.

CALCULATION OF INITIAL CONCENTRATIONS. To calculate the initial concentrations of the two types of particle in the centrifuge tubes, it was assumed that the concentrations at a point chosen near the bottom of the tube were related to the initial concentration by a formula which applies to a gradient that is linear in density only (Gibson, 1965b). This formula is

$$c_0 = ce^{\alpha\beta\omega^2 t} \left\{1 - \frac{x}{\alpha}(1 - e^{-\alpha\beta\omega^2 t})\right\}^2 \qquad (3)$$

where α is a function of the densities $\rho(x_1)$ and $\rho(x_2)$ at the coordinates of the meniscus (x_1) and the bottom of the tube (x_2) and of the density ρ_0 of the sedimenting material. The quantity β is given in terms of η, d, and q by

$$\beta = \frac{d}{18q\eta} \times \frac{\rho(x_2) - \rho(x_1)}{x_2 - x_1} \qquad (4)$$

Values were calculated for α and β and substituted in eq 3 to obtain c_0 in terms of the experimentally determined values of c and x.

Estimation of Polydispersity. To estimate the degree of polydispersity of the chromatophores, moving boundary sedimentation was performed in a reciprocal density gradient (Gibson, 1965b). The gradient was constructed from solutions of KCl in such a way as to make the density, in g/cm^3, satisfy the relation

$$\rho(x) = 1.175 - 0.928/x \qquad (5)$$

where x is the coordinate measured from the centrifugal axis in centimeters. The gradients were produced either by layering solutions of KCl at the appropriate concentrations, the volumes being adjusted so that each layer was 0.2 cm deep, or by means of a one-dimensional apparatus constructed according to Bock and Ling (1954). The apparatus was designed so that the coordinate of the meniscus (x_1) was 5.3 cm and that of the bottom of the gradient (x_2) was 9.3 cm; the space below this was filled with a solution at the same density as that at the bottom of the gradient. Centrifugation was carried out as described above, except that all runs were performed at 35,300 rpm and the contribution of acceleration and deceleration was calculated directly from the time integral of the square of the angular velocity. After each run the contents of the tube were analyzed by passing them through the Autoanalyser and determining the optical density at 440 mμ.

A particle whose density is 1.175 g/cm^3 has a constant velocity of sedimentation in the gradient of eq 5, which can be written as $\mu\omega^2$; here ω is the angular velocity and μ is related to η, d, and q by

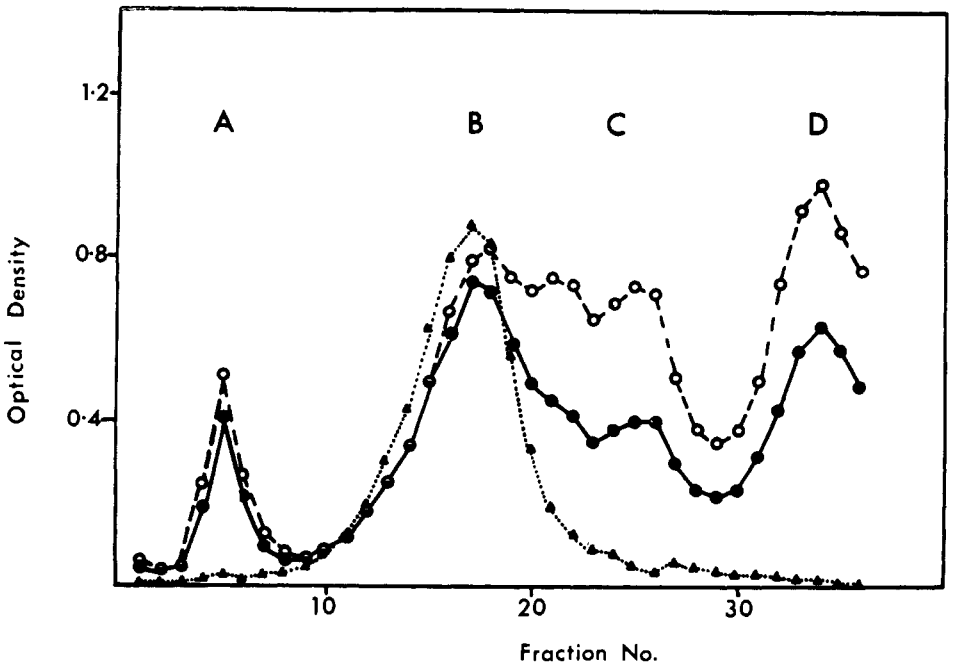

FIGURE 1: Zone centrifugation of extract of anaerobic cells on a linear CsCl gradient. Fractions were collected from the bottom of the tube and their optical densities determined at 260 (O—O), 280 (●—●), and 850 mμ (▲···▲). For the significance of peaks A–D see the text.

$$\mu = \frac{d^2}{18q\eta} \times \frac{x_1x_2\{\rho(x_2) - \rho(x_1)\}}{x_2 - x_1} = 0.0513\frac{d^2}{q\eta}\ \text{sec}^{-1} \tag{6}$$

where d and η are expressed in cgs units. The mean and variance of the distribution of μ were calculated from the experimentally observed distribution of the pigment by means of the formulas (see Appendix)

$$\bar{\mu} = \frac{1}{\omega^2 t}\left\{x_2 - x_1 - \frac{1}{c_p}\int_{x_1}^{x_2} c(x)\mathrm{d}x\right\} \tag{7}$$

$$\sigma^2(\mu) + \bar{\mu}^2 = \frac{1}{\omega^4 t^2}\left\{(x_2 - x_1)^2 - \frac{2}{c_p}\int_{x_1}^{x_2}(x - x_1)c(x)\mathrm{d}x\right\} \tag{8}$$

where c_p is the concentration in the plateau region.

The variance calculated from these equations represents a maximum value, since the method of sampling in the Autoanalyser tends to make the boundary appear more diffuse than it actually is. To allow for this, an artificial boundary was made by layering a clear solution over a more dense solution containing pigment and the mixture was passed through the Autoanalyser. A value of the variance σ_s^2 of the error introduced by the sampling techniques was calculated from the observed distribution of pigment by using eq 7 and 8. The only remaining factor which might contribute to the observed distribution of materials is diffusion; however, an approximate calculation showed that the variance of the error caused by diffusion was smaller than σ_s^2 by a factor of 10^3, and it was therefore neglected. If the contribution to the spreading of the observed boundary made by the method of sampling were independent of the contribution which comes from the actual distribution of μ, the true variance of μ would be related to the observed variance by

$$\sigma^2(u) = \sigma_{obsd}^2 - \sigma_s^2 \tag{9}$$

This equation was used to obtain a corrected value for $\sigma^2(\mu)$ which represents the smallest possible value consistent with the variance of the observed distribution of pigment.

Assay Procedures. Protein was estimated either by the method of Warburg and Christian (1941) or by that of Lowry *et al.* (1951) with crystalline bovine serum albumin (Armour) as standard. Bacteriochlorophyll was estimated according to Cohen-Bazire *et al.* (1957).

Results

When an extract of light-grown *Rps. spheroides* is centrifuged in a gradient of CsCl only a single peak containing pigment is obtained, as judged by the absorption at 850 mμ (Figure 1). If the optical densities at 260 and 280 mμ are also determined several other peaks can be discerned. These peaks are distinguished by the ratio (OD at 260 mμ)/(OD at 280 mμ), which takes values of about 1.2 for the lowest peak (A, Figure 1), 1.0 for the peak containing the pigment (B, Figure 1),

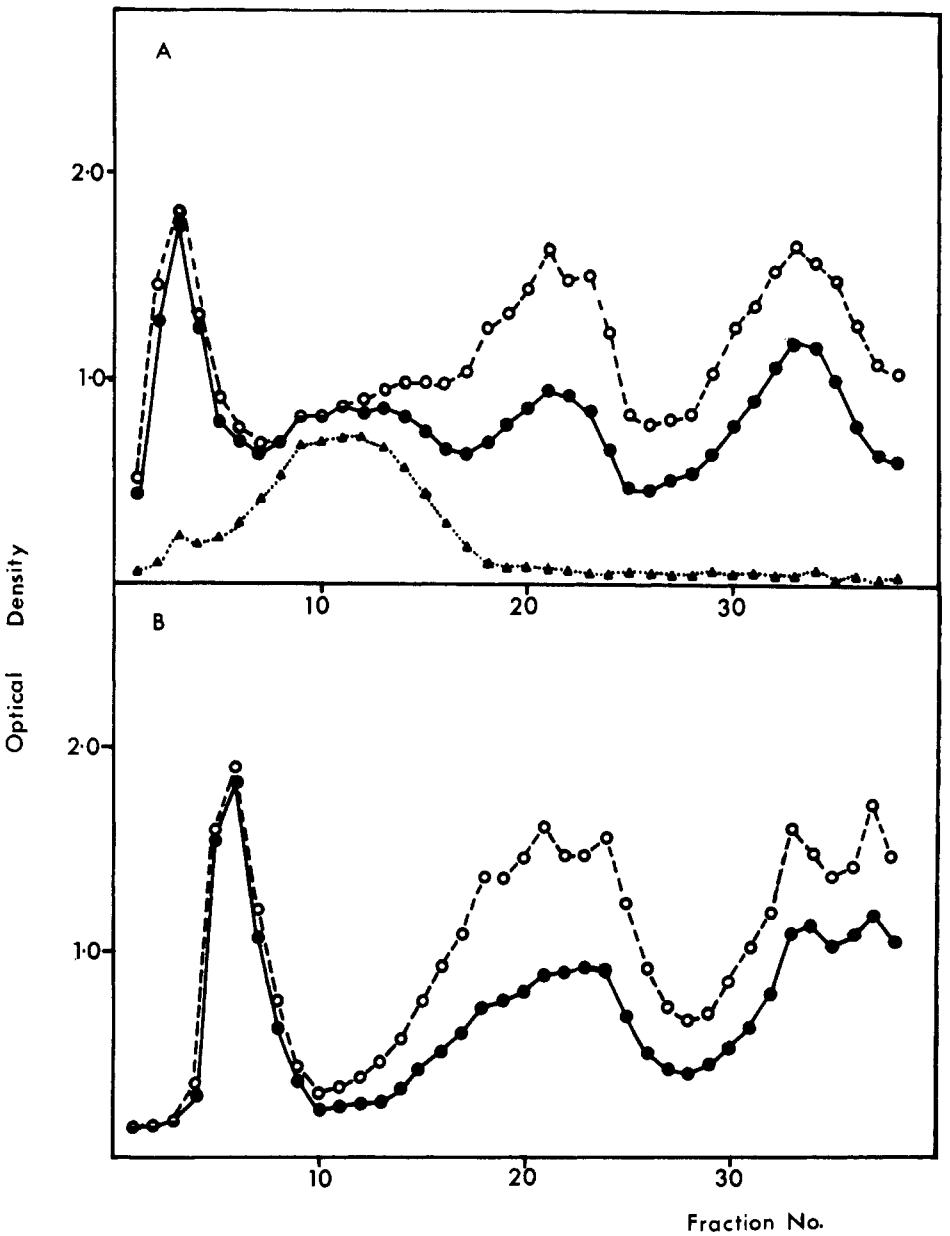

FIGURE 2: Zone centrifugation of extracts on a linear CsCl gradient. Experimental procedure and wavelengths as in Figure 1; 260 (○), 280 (●), and 850 mμ (▲). (A) Anaerobic cells harvested in the middle of the log phase. (B) Strictly aerobic cells.

and 1.6–1.8 for the remaining peaks (C, D in Figure 1). The apparent homogeneity of the peak containing the pigment raises the question whether the separation is due to density alone or is influenced by size and shape also; that is, whether the peaks contain particles with a variety of different sizes but having all the same density. It was found that the pigment migrated as a fairly narrow band for at least 120 min and that at the end of this period it was still moving; this was in contrast to peak A which reached its final position in the CsCl gradient within 45 min. Thus, although peak A separated on the basis of density alone, the movement of the pigmented particles in these gradients was influenced by size and shape also.

In these experiments an appreciable quantity of pigment passed completely through the CsCl gradient and was precipitated with the pellet at the bottom of the tube. It was also found that up to 20% of the pigment in a crude extract of cells could be precipitated by centrifugation for 10 min at 10,000 rpm in an angle-head centrifuge. These observations suggested that an important part of the pigment might be attached to large cell fragments, as would be expected if the chromatophores were part of a reticular structure which was con-

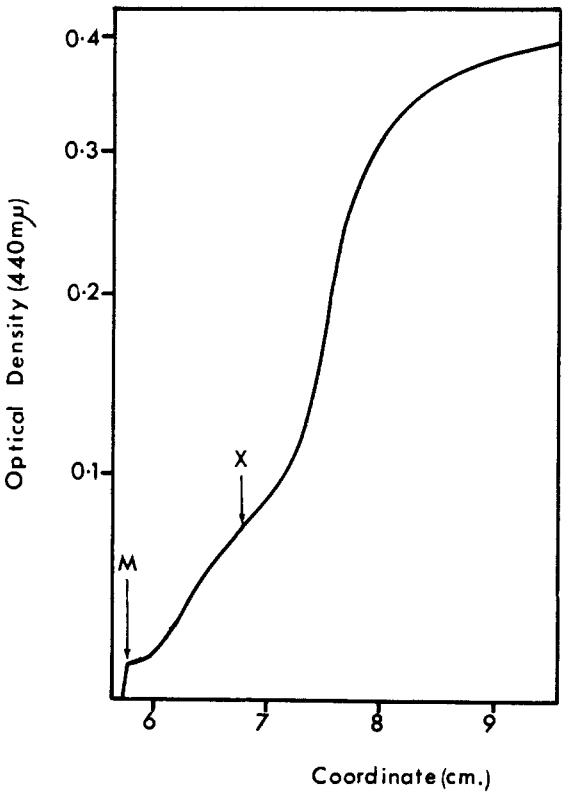

FIGURE 3: Moving boundary sedimentation of extract of anaerobic cells. Linear KCl gradient (2.9–6.7% (w/w)), 33,500 rpm, 42.0 min, 3.8°. Contents of tube sampled from the top; M, meniscus; X, point of division for calculating positions of boundaries.

tinuous with the cytoplasmic membrane. To test this point, an extract made by passing a suspension of organisms through the French press and containing about 50 mg of protein/ml was passed through the press twice more, and after each passage an aliquot (0.15 ml) was centrifuged through a CsCl gradient and the supernatant liquid was removed and fractionated. Figure 1 shows the distribution of the various peaks in one of these extracts. The pattern presented by the other two extracts was indistinguishable from this one, and in particular there was only one pigmented peak in each case. The pellets remaining in the centrifuge tubes were resuspended and their pigment content determined from the optical densities at 850 mμ. The fractions of the total bacteriochlorophyll layered on the gradient which were found in the pellet at the end of the experiment were 20% for the extract obtained by crushing the cells once, 8% for that obtained by two crushes, and 4% for the extract obtained by crushing three times. Thus repeated passage through the French press liberated more and more pigmented particles from their attachment to large cell fragments without apparently causing them to break down into smaller pieces, and at least 95% of the total pigment in the cell could be made to appear as a single rather narrow band in the centrifuge.

When an extract of aerobically grown cells was centrifuged in a CsCl gradient the same pattern of ultraviolet absorbing peaks was obtained as in anaerobic cultures, except that the pigmented peak was absent (Figure 2b). Anaerobic cultures which were harvested in the middle of the log phase (Figure 2a) showed patterns similar to those obtained with cultures harvested at the end of growth (Figure 1), except that the pigmented peak was somewhat smaller and the ratios of the optical densities at 260, 280, and 850 mμ were slightly different. Figure 2 suggests strongly that as far as protein-containing particles are concerned the only difference between aerobically and anaerobically grown *Rps. spheroides* is that the latter contain pigmented particles which are absent from the former. An extract of a culture grown aerobically at low oxygen tension showed a curve intermediate between those in Figures 2a and 2b.

The nature of the material in the various peaks is still under study. The purification and properties of the pigmented particles in peak B are described in the accompanying paper (Gibson, 1965a). From its absorption in the ultraviolet region and from preliminary chemical analysis and examination in the electon microscope, together with an approximate calculation of the sedimentation coefficient, it is concluded that peak C consists mainly of ribosomes. Peak D appears to contain the soluble protein and nucleic acid of the cell. Examination in the electron microscope, chemical analyses, and experiments with labeled acetate have shown that peak A contains poly-β-hydroxybutyrate when this is present, some material from the cell wall or cytoplasmic membrane, possibly some membrane-bound vesicles; and indeed it seems to be a mixture of fragments from most of the larger structures in the cell. However, the bulk of the cell walls are found in the pellet at the bottom of the tube (unpublished experiments).

Moving Boundary Sedimentation. The results presented above suggested that the majority of the pigment of *Rps. spheroides* might be contained in a single particulate fraction. However, it was also possible that some sort of aggregation might have taken place or that the resolution of the CsCl gradients was not sufficient to separate particles of similar sizes. In an attempt to overcome these difficulties a series of moving boundary centrifugation experiments were performed in the preparative ultracentrifuge using the absorption at 440 mμ due to carotenoid as a measure of the pigment. The organisms were crushed by one to three passages through the French press, and the bulk of the unbroken cells and larger cell fragments were removed by centrifugation for 10 min at 10,000 rpm in an angle-head centrifuge. This procedure also removed between 5 and 10% of the pigment. Aliquots of the supernatant were diluted with 0.01 M Tris buffer until the protein concentration was 0.5–2 mg/ml and were then centrifuged in gradients of KCl as described in Materials and Methods. The concentration of pigmented particles in these experiments was judged from the absorption at 440 mμ to amount to 0.05–0.2 mg of protein/ml using a factor calculated from the composition of purified particles.

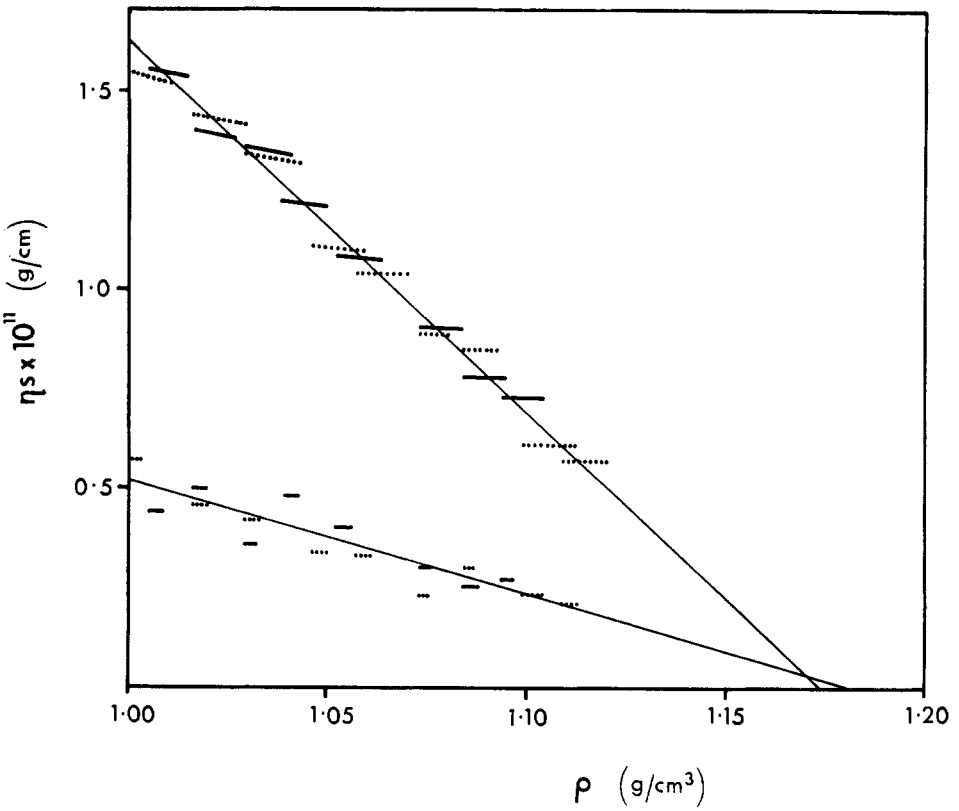

FIGURE 4: Plot of viscosity × sedimentation coefficient (ηs) against density (ρ). Experiments 1 (.....) and 2 (———) were performed with different extracts. Lines are lines of regression through midpoints of intervals.

The result of a typical run in which the contents of the tube were run into the Autoanalyser from the top is shown in Figure 3. Whichever method of sampling was employed there were two boundaries, and the same conclusion was reached from visual inspection of the tubes before sampling.

In order to characterize the particles corresponding to the two boundaries a series of centrifugal runs were performed at different densities by varying the average concentration of KCl in the tube. Two groups of experiments were performed in each of which the average concentration of KCl was varied over the range 2–18% (w/w). The extract used in the first group of experiments was obtained by a single passage through the French press, whereas that used in the second group was passed through it three times. The tubes were sampled from the bottom in the first group and from the top in the second. In both cases the curves were analyzed in terms of two boundaries, as discussed in Materials and Methods. The s values obtained by means of eq 1 were plotted according to eq 2, and the result is shown in Figure 4. It is clear that the results were completely consistent in spite of the variations in technique. The use of eq 2 carries with it the assumption that the particles do not behave as osmometers when the salt concentration is varied (de Duve *et al.*, 1959), but as no evidence has been brought forward to suggest that bacterial chromatophores have a permeability barrier to inorganic salts this assumption appears justified. As demonstrated elsewhere the value for s calculated from eq 1 is equal to the value which the sedimentation coefficient actually takes at some point between the initial and final positions of the boundary (Gibson, 1965b). In Figure 4 a line has been drawn for each value of ηs to demarcate the corresponding interval of values of ρ determined by the initial and final positions of the boundaries. Since the viscosity also varied slightly with the concentration of KCl the lines do not all lie parallel to the abscissa. The densities of the particles were obtained from the intercepts of the lines of regression of ηs on ρ, using the values assumed by ηs at the left-hand end points, midpoints, or right-hand end points of the intervals. The slopes of these lines were used to calculate the quantity $dq^{-1/2}$, where d is the diameter of the equivalent hydrodynamic sphere and q is the frictional ratio; the problem of evaluating d and q is discussed below. The lines drawn in Figure 4 are the lines of regression calculated for the midpoints of the intervals.

The values for $dq^{-1/2}$, ρ_0, and $s_{20,w}$, together with the correlation coefficient between ηs and ρ, deduced from each line of regression, are shown in Table I. There is some degree of choice in the values obtained for the density and $s_{20,w}$ value of the large particles; however, the uncertainty introduced by the method of calculation is probably no greater than the experimental error. There is rather less doubt about the value of $dq^{-1/2}$

TABLE I: Parameters of Equivalent Hydrodynamic Spheres.

Relative Size of Particles	Line of Regression through	Correlation Coefficient	$s_{20,w}$	$dq^{-1/2}$ [a] (A)	Density[b] (g/cm³)
Large	Left-hand end points	−0.995	156	409	1.169
	Midpoints	−0.995	161	410	1.174
	Right-hand end points	−0.995	165	406	1.181
Small	Midpoints	−0.915	52	227	1.181

[a] From slope of line of regression. [b] From intercept of line of regression on abscissa.

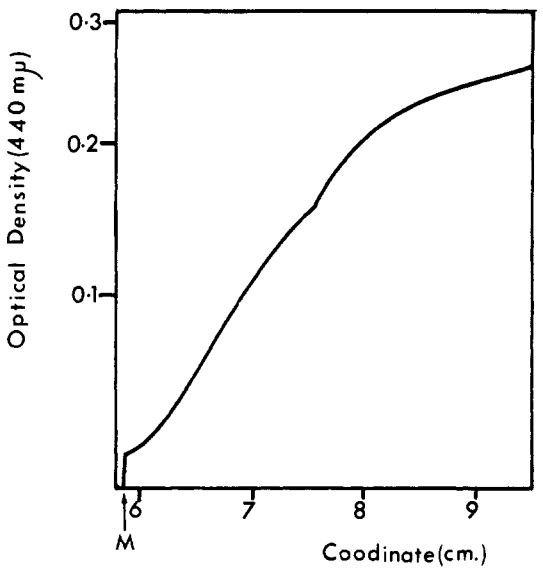

FIGURE 5: Moving boundary sedimentation of extract of semi-aerobic cells. Linear KCl gradient (0–3.8% (w/w)), 35,400 rpm, 30.0 min, 9.8°. Contents of tube sampled from top; M, meniscus.

for the equivalent hydrodynamic sphere, which is close to 410 A (the figure given in the preliminary communication is erroneous). The correlation coefficient between ηs and ρ for the large particles is very close to −1.0 whichever set of values of ρ was chosen (Table I). On the other hand there is not such a striking degree of consistency in the results obtained for the small particles, and the values for $dq^{-1/2}$, ρ_0, and $s_{20,w}$ of these particles must be regarded as approximate. The occurrence of greater errors in the location of a boundary for the smaller particles is to be expected in view of their smaller concentration and of the fact that although they did not migrate very far down the tubes during the centrifugal runs no allowance was made in locating a boundary for deviations from a plateau. Also they are plainly much more polydisperse than the 160 S particles. The 55 S particles were not detected readily in the experiments with zone centrifugation, since they contain little pigment and are found in the same peak as the much more numerous ribosomes. However they could be seen as a faint band of color lying above the main pigmented band when some extracts were examined by zone centrifugation.

The relative amounts of the two types of particle were determined approximately by means of eq 3. For this purpose the values of d^2/q and ρ_0 deduced from the midpoints of the intervals in Figure 4 were used. The calculations gave a range of values of initial concentrations whose means and standard deviations for the two groups of experiments are shown in Table II. Clearly

TABLE II: Relative Concentrations of 55 S and 160 S Particles.

	Estimates of Initial Concentration of 160 S Particles		of 55 S Particles	
Expt.	(SD[a]) Mean	Mean[b]	(SD[a]) Mean	Mean[b]
1	0.078	82	0.083	18
2	0.042	86.5	0.082	13.5

[a] SD, standard deviation. [b] As per cent of sum of means.

the values obtained for the relative concentrations of the particles would depend very markedly on the choice of the point X in Figure 3. The calculations would also be in error if the gradients in which centrifugation was carried out deviated significantly from linearity, or if either type of particle were very polydisperse. However none of these factors will introduce such large errors into the determination of s values. For these reasons the figures in Table II are liable to more error than those in Table I; nevertheless the data in Table II indicate that in these two extracts the large particles accounted for about 85% of the pigment in the extracts and the small ones for the remaining 15%. It was found that the fraction of pigment present in small particles varied considerably among different cultures of organisms. In

anaerobically grown organisms the fraction of pigment associated with small particles was seldom higher than 15% and was frequently almost zero. With cultures grown semi-aerobically in the dark, at oxygen tensions which were sufficiently low to allow the formation of bacteriochlorophyll (Lascelles, 1959), a larger proportion of pigment was found to be associated with small particles. The distribution of pigment in an extract of one such culture which contained 2.4 μmmoles of bacteriochlorophyll/mg dry weight is shown in Figure 5. Two boundaries are visible whose s values were 53 and 152 S when corrected to 20°, agreeing well with the values obtained for anaerobic organisms. However, calculation of the relative initial concentrations of pigment in the two types of particle showed that about 50% of it was present in each type. It must be emphasized that all these estimates are based on absorption in the region of carotenoid absorption, which does not reflect exactly the concentration of bacteriochlorophyll. Preliminary experiments with moving boundary sedimentation, in which the contents of the tubes after centrifugation were divided manually into several fractions, showed that, although the absorption at 440 mμ closely paralleled that at 850 mμ with both the 55 and the 160 S particles, the carotenoid absorption was slightly higher in the 55 S particles relative to the bacteriochlorophyll absorption than it was in the 160 S particles.

Degree of Polydispersity of 160 S Particles. An estimate of the degree of polydispersity of the 160 S particles was made with the aid of the reciprocal density gradient described in eq 5. The extracts which were used for these experiments were centrifuged at 10,000 rpm for 10 min before use. Provided that the 55 S particles were absent, or at least unpigmented, the analysis by means of eq 7 and 8 was straightforward. The distribution of pigment obtained with one such preparation is shown in Figure 6, and the mean together with the maximum and minimum values for the variances of μ calculated from this experiment and a similar one are given in Table III. In one of the experiments in this table the gradient was made by means of a specially constructed apparatus while in the other it was made by layering solutions on top of one another. The agreement between the two is very satisfactory. The figures in Table III are calculated on the assumption that all the particles have the same density. If the particles vary in density as well as diameter the values for the variance of μ given in the table will be greater than the true values. It was found that the plateau concentration in one of these experiments was the same when the time of centrifugation was 30.5 min as it was when the time was 7, 12, or 20 min; this confirms that the mean density of the particles was 1.175 g/cm³ since otherwise the boundary would not migrate at constant speed and the concentration would change. Preliminary experiments using equilibrium density centrifugation suggested that the particles do not have widely distributed densities.

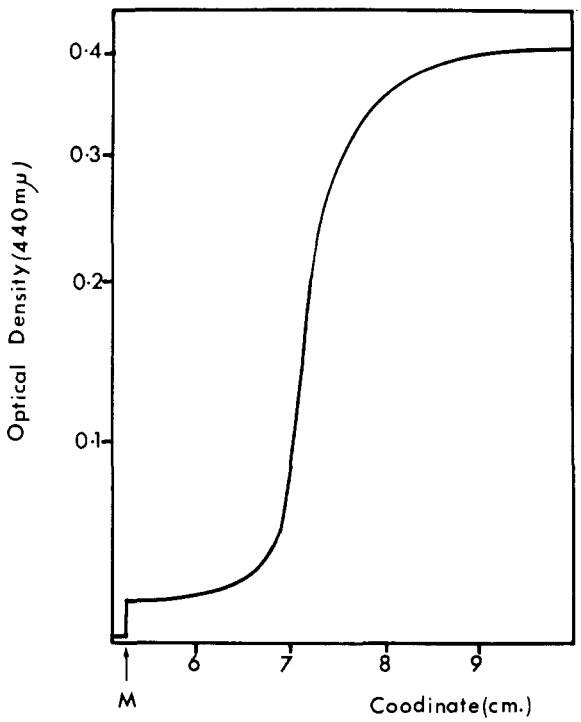

FIGURE 6: Moving boundary sedimentation in reciprocal gradient of KCl. Extract of anaerobic cells; 35,300 rpm, 33.8 min, 7°. Contents of tube sampled from the top; M, meniscus.

TABLE III: Mean and Variance of μ for 160 S Particles.

Expt	Temp (°C)	μ (sec^{-1} $\times 10^{11}$)	$\sigma^2(\mu)$ (sec^{-2} $\times 10^{21}$)	$\sigma_s^{2\,a}$	$\sigma^2(\mu) - \sigma_s^2$
1	2.2	5.45	7.2	2.7	4.5
2	7	6.50	8.3	2.1	6.2

[a] Variance of the spreading due to the method of sampling (see Materials and Methods).

Equation 6 shows that μ is proportional to d^2/q. The mean and variance of the quantity $dq^{-1/2}$ can be derived from the distribution of μ by making a suitable assumption about the form of the distribution of $dq^{-1/2}$. The simplest assumption is that q is constant and d is normally distributed; then the mean and standard deviation of the distribution of d can be calculated up to the constant factor $q^{-1/2}$ from the mean and variance of the distribution of μ. This has been done in Table IV, using the data of Table III. There is good agreement between the values obtained for the two experiments, which represent different cultures of organisms; and there is also good agreement between the values for the root-mean-square value of $dq^{-1/2}$ calculated from $\mu^{1/2}$ and the value given in Table I (which ought to be close to the root-mean-square value). The figures given in Table IV show that if the assumptions are correct the quantity $dq^{-1/2}$ for the hydrodynamic spheres equivalent to the

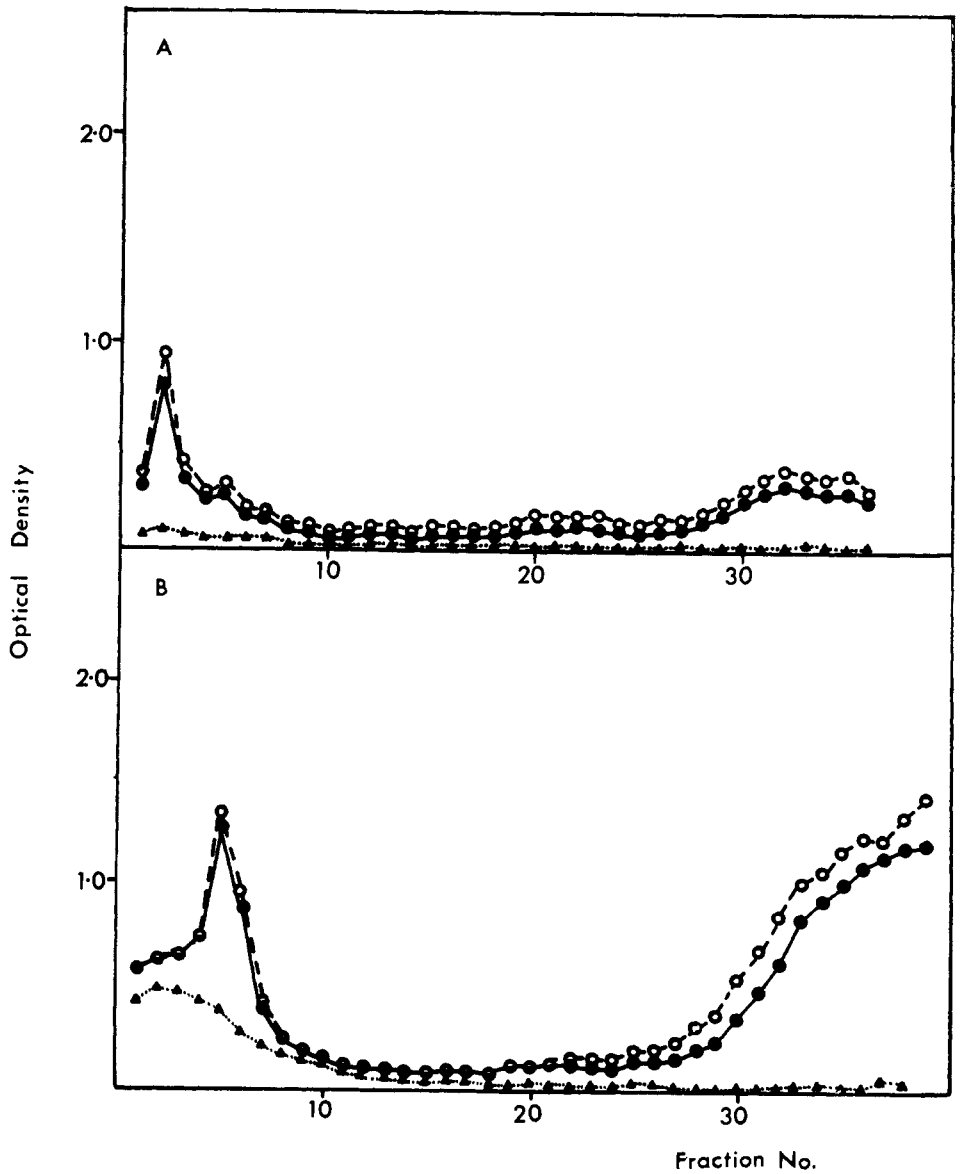

FIGURE 7: Zone centrifugation of lysozyme–EDTA extracts of anaerobic cells. Experimental procedure and wavelengths as in Figure 1; 260 (○), 280 (●), and 850 mμ (▲). (A) Extract prepared by lysis alone. (B) Extract prepared by lysis and passed through French press.

160 S particles has a mean value of about 395 A and a standard deviation which is at least 35 A but not greater than 50 A. Evidence which supports these assumptions is advanced in the Discussion.

Distribution of Pigment in Lysed Preparations. The results presented above indicate that in anaerobically grown *Rps. spheroides* the greater part of the pigment is contained in particles whose mean sedimentation coefficient is about 160 S and whose diameters do not vary by more than about 12%. This is in marked contrast to the observations discussed in the introductory section which showed that when cells of *R. rubrum* are converted to spheroplasts and then lysed all the pigment remains attached to much larger cell fragments. It was therefore important to investigate the distribution of pigment in cells which had been ruptured in this way.

Spheroplasts of *Rps. spheroides* were produced by treatment with lysozyme and EDTA in the presence of sucrose, and lysed by osmotic shock. The pigment in the resulting extracts could be completely sedimented by a short low-speed centrifugation (*e.g.*, 10,000 rpm for 5 min) just as was observed by Gest and his collaborators with *R. rubrum*. When aliquots of such extracts were layered on top of a CsCl gradient and centrifuged in the usual way, all the pigment was recovered in the pellet at the bottom of the tube. However, the distribution of material in other peaks was altered as well; in particular the peak which contains ribosomes (peak C in Figure 1) was absent although the heaviest and lightest peaks (A and D in Figure 1) were present

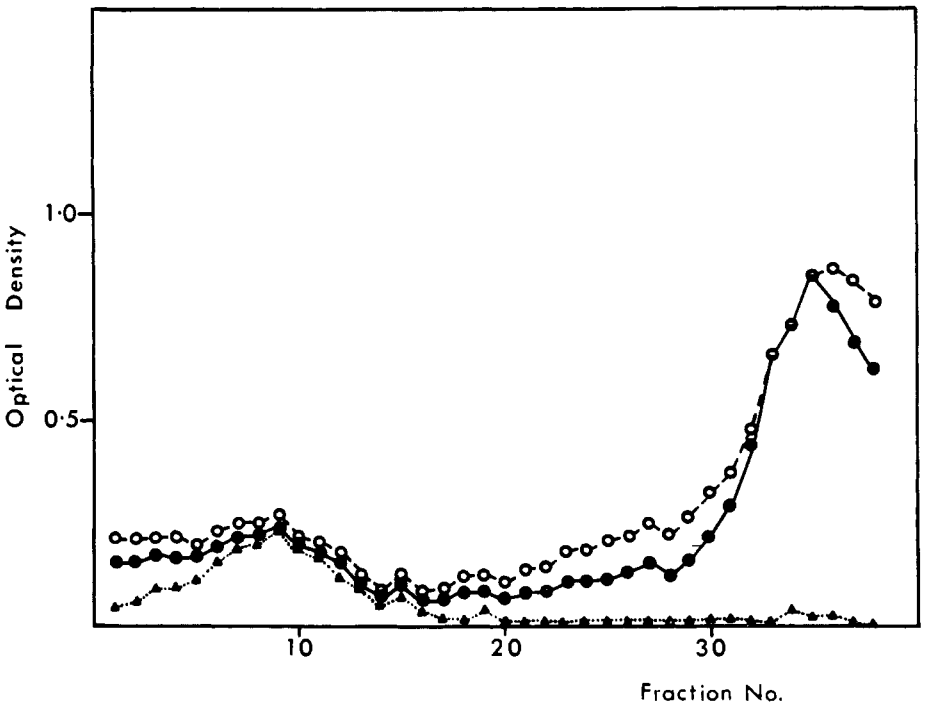

FIGURE 8: Zone centrifugation of lysate of ampicillin-treated anaerobic cells. Experimental conditions and wavelengths as in Figure 1; 260 (○), 280 mμ (●), and 850 mμ (▲).

TABLE IV: Calculated Mean and Standard Deviation of Diameters of 160 S Particles.[a]

Expt	Values with Max Variance[b] $d/q^{1/2}$	$\sigma(d)/q^{1/2}$	Values with Min Variance[c] $\bar{d}/q^{1/2}$	$\sigma(d)/q^{1/2}$	Root-Mean-Square[d] Value of $d/q^{1/2}$
1	378	47	394	35	405
2	394	42	403	36	415

[a] Calculated from data in Table III and expressed in A. [b] Calculated from $\bar{\mu}$ and $\sigma^2(\mu)$. [c] Calculated from $\bar{\mu}$ and $\sigma^2(\mu) - \sigma_s^2$. [d] Calculated from $\bar{\mu}$.

in their usual positions but in decreased amount (Figure 7a). Omission of $MgCl_2$ from the method of preparation, or alteration of the pH during the treatment with lysozyme to 7.4 or 8.0, did not change the distribution of material in the CsCl gradient.

A lysate prepared from lysozyme–EDTA treated cells was passed through the French press and an aliquot was then layered onto a CsCl gradient and centrifuged. No material was found in the positions normally occupied by peaks B and C; on the contrary the only pigmented particles which were released migrated below peak A (Figure 7b), indicating that they were more aggregated than the particles which are released when cells are ruptured with the French press alone. Even when the lysate was passed through the French press twice more no material corresponding to peaks B or C was released, but some more pigmented particles were found in the new position below peak A. This result is not what would be expected if the pigmented particles arise by comminution of a structure which is released intact by lysis of spheroplasts, but suggests that the treatment with lysozyme and EDTA had altered the internal structure of the cell. For this reason alternative methods of lysis were investigated.

One technique which has been used successfully to lyse Gram-negative organisms is to convert them to spheroplasts by growing them in the presence of benzylpenicillin (see McQuillen, 1960) or 1,5-dimethoxyphenylpenicillin (methicillin) (Aldrich and Sword, 1964) and then to treat the spheroplasts with lysozyme. Neither of these antibiotics can be used with *Rps. spheroides* as it appears to be able to grow in the presence of benzylpenicillin at a concentration of 2 mg/ml or methicillin at 0.5 mg/ml. However the organism was found to be sensitive to ampicillin at concentrations of 30 μg/ml, and it appeared possible to produce spheroplasts with this substance. When these were lysed with lysozyme about 95% of the pigment (measured by the absorption at 850 mμ) was released in a form which was readily sedimented by brief low-speed centrifugation, in a manner analogous to the material released from lysozyme–EDTA spheroplasts. Approximately the same proportion of the pigment was recovered from the pellet at the bottom of the tube when an aliquot of the

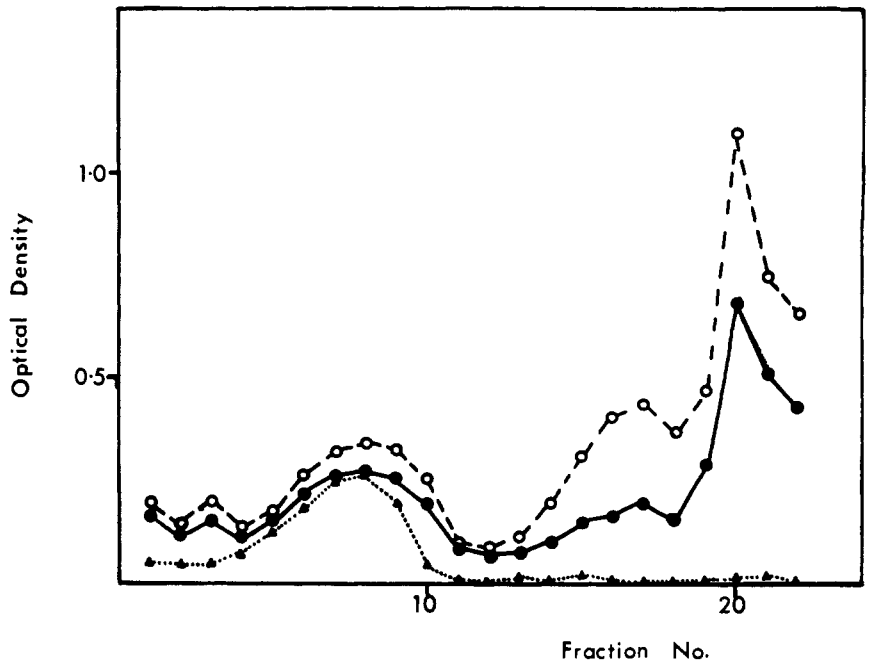

FIGURE 9: Zone centrifugation of lysozyme–polymyxin B extract of anaerobic cells after treatment in a blendor. Experimental conditions and wavelengths as in Figure 1; 260 (○), 280 (●), and 850 mμ (▲).

lysate was layered on a CsCl gradient and centrifuged. However the small amount of pigment which did not reach the bottom of the tube was found as a narrow band in the same position as peak B from a French press extract (Figure 8), and there was also a small amount of peak C material released. A lysate produced by the action of ampicillin and lysozyme was passed through a French press. The resulting extract gave rise to a profile resembling those in Figure 1 when it was layered onto a CsCl gradient and centrifuged. Identical results were obtained when lysis was carried out by treating cells with lysozyme and polymyxin B.

An explanation of these observations is that the pigment is present in particles which are held together firmly in the cell but can be separated by applying shearing forces. If this is true it should be possible to release pigmented particles of the same size as those released in the French press by subjecting lysates of spheroplasts to other types of shearing force. This possibility was investigated with a lysate produced through the action of lysozyme and polymyxin B. The lysate was first homogenized in a Potter–Elvehjem homogenizer with a Teflon pestle for about 3 min. When an aliquot of the homogenate was layered onto a CsCl gradient and centrifuged, about 85% of the pigment was in the pellet; however, the other 15% was all recovered in a narrow band at the same position as peak B in Figure 1. Thus homogenizing the lysate in a Potter–Elvehjem apparatus released about 10% of the pigment as particles of the same size as those released in the French press. When the lysate was homogenized in a small blendor (Atomix, Measuring and Scientific Equipment Co., Ltd., London) for 3 min about 25% of the pigment appeared in the position of peak B after centrifugation through a CsCl gradient, the remaining 75% being associated with the pellet. The distribution of material among the pigmented and other particulate fractions in the CsCl gradient was essentially the same as was found with French press extracts (Figure 9).

Discussion

The results of the various experiments reported here all favor the original suggestion of Schachman *et al.* (1952) that at least in some of the *Athiorhodaceae* the photosynthetic pigments are contained in discrete particles rather than a continuous structure. The observation that at least 95% of the pigment can be released in a form which migrates mainly in a single peak when subjected to zone centrifugation in a CsCl density gradient suggests that the pigment is almost all contained in the same type of subcellular structure, which is composed of rather uniform particles, or at least breaks up into such particles when subjected to strong shearing forces. The results of moving boundary sedimentation confirm that at least 85% of the pigment present in extracts of anaerobic cells is contained in a rather homogeneous particulate fraction whose $s_{20,w}$ value is 160 S. The significance of the 55 S particles that contain the rest of the pigment is not clear. In anaerobic organisms they do not account for more than 15% of the photosynthetic pigments, although there appeared to be much more pigment in small particles from organisms which were grown under semi-aerobic conditions. If the 55 S particles were all fragments released from the 160 S particles by comminution in the

French press there would have to be a marked difference in the stability of the 160 S particles from aerobic and anaerobic cells. On the other hand there is at present no direct evidence to suggest that the small particles are precursors of the large ones.

Polydispersity of 160 S Particles. Additional evidence in favor of the view that the pigment is contained in particulate structures *in vivo* is provided by the narrowness of the distribution of particle diameters. On the assumption that the diameters of the equivalent hydrodynamic spheres are normally distributed it was found that the standard deviation of this distribution was not more than 12% of its mean. Unless there is a very great variation in the degree of hydration of the particles there should be an equally narrow distribution for the diameters of the hydrated particles; indeed, as discussed below, an analysis of electron micrographs of purified particles showed that this is so (Gibson, 1965c). If the particles which are present in extracts are formed by comminution of a continuous membranous structure they might be expected to have a broader distribution of sizes by analogy with the microsomes formed by the reticular endothelium of liver and of pancreas (Palade and Siekevitz, 1956). The sizes of those microsomes were estimated from electron micrographs, and their range may therefore appear greater than if estimated from sedimentation data; but even allowing for this the microsome fraction is undoubtedly more polydisperse than the 160 S particles from *Rps. spheroides*. It seems that if the 160 S particles are derived from a continuous structure it must be of such a nature that it readily breaks down into particles of very similar sizes which apparently retain their original biochemical function. As the structure also looks particulate when viewed in the electron microscope it is difficult to avoid the conclusion that it actually consists of particles, which may be held together in clumps inside the cell but are essentially independent and functionally distinct structures.

Although it is clear that the 160 S particles are not very polydisperse, nevertheless the evidence indicates that they are by no means monodisperse. Table IV gives a minimum value for the standard deviation of the diameters, calculated on the assumption of a normal distribution, of about 9% of the mean. This is not as large as the variation in size of mitochondria (Thomson and Klipfel, 1957; Bahr and Zeitler, 1962); nevertheless it shows that the 160 S particles are not uniform in size like virus particles.

Frictional Ratio and Hydration of 160 S Particles. It is shown elsewhere by direct examination of electron micrographs that the 160 S particles are spheres or ellipsoids whose axial ratio is less than 1.4 (Gibson, 1965c). The diameters of the particles are distributed approximately normally about a mean of 570 A, with standard deviation equal to 7% of the mean. In Table IV the diameters of the equivalent hydrodynamic spheres were found to have a standard deviation which was 9–12% of the mean if it was assumed that these diameters are normally distributed and that each particle is hydrated to the same extent. If these assumptions are justified the ratio (standard deviation/mean) should be the same for the whole particle and its equivalent sphere. The difference between the observed ratios is probably within experimental error, so the hypothesis is at least a very good approximation. The degree of hydration can be calculated by observing that for particles which are almost spherical the frictional ratio q is very nearly equal to D/d, where D is the diameter of the hydrated particle. Thus the quantity $\bar{d}^2/q$ is equal to $\bar{d}^3/D$. Taking the mean value D to be 570 A and the mean value $\bar{d}/q^{1/2}$ to be 395 A, the value of $\bar{d}$ is found to be 435 A. Hence the frictional ratio q is 1.31 and the amount of water bound to the particle is equal to 1.05 g/g dry weight, or 55% of the total volume (these values replace the approximate figures deduced elsewhere from preliminary data (Gibson, 1964b)). From these figures the mean molecular weight is calculated to be 3.0×10^7 for the equivalent sphere, or 6.2×10^7 for the fully hydrated particle.

Distribution of Pigment in Lysates. The results presented here suggest that the conversion of cells into spheroplasts by means of lysozyme and EDTA can be accompanied by changes in the internal components of the cell. It is clear from Figure 7 that this treatment causes both the pigmented particles and the ribosomes to aggregate, and this aggregation is not significantly reversed by passing the lysate through a French press. It is difficult to avoid concluding that the treatment with lysozyme and EDTA may irreversibly alter the internal structure of the organism in such a way as to cause permanent aggregation of the subcellular particles. In fact it is quite possible that the structures, which have been observed in the electron microscope to connect chromatophores in lysozyme–EDTA spheroplasts and lysates of *R. rubrum* (Boatman, 1964), were artifacts introduced during the conversion of cells to spheroplasts.

On the other hand, when *Rps. spheroides* was lysed without the use of EDTA and sucrose there was no irreversible change in either the pigmented particles or the ribosomes, and the results of these experiments must be taken into account when the state of the pigment *in vivo* is discussed. Some of the pigment (about 5%) was liberated by these methods of lysis in particles of the same size as those in which the bulk of the pigment is recovered in French press extracts; these are in fact the 160 S particles. However the remainder of the pigment was found in much larger structures which sedimented readily and could only be released by shearing forces. The question therefore arises whether the particles are artifacts caused by the application of shearing forces, or whether on the contrary the fraction which accounts for the majority of the pigment consists of particles which are held together by noncovalent forces and which separate when a shearing force is applied. If the former were true it might be expected that different types of shearing force would produce different sizes of particles, whereas the latter hypothesis should produce the same size of particles however the shearing force is applied. It was found that the particles released by applying shearing forces to the lysate were always of the

same size. This was true whether the forces were applied as a single sharp impulse (in a French press), as mild forces over a longer period (in a Potter–Elvehjem homogenizer), or as forces of varying intensity (in a blendor). These observations support the view that the structure which contains the bulk of the pigment in the lysates consists of aggregates of the 160 S particles. Further support for this view comes from the readiness with which purified chromatophores aggregate (Gibson, 1965a).

However, even if these conclusions are incorrect, there are two significant points to be drawn from the experiments with lysates discussed here. The first is that it is possible to put forward hypotheses, and to provide some experimental support for them, which offer an explanation for the results without contradicting the view that the photosynthetic pigments are contained in particulate structures. The second point is that there appears from Figures 7, 8 and 9 to be a close correspondence between the release of 160 S pigmented particles and of ribosomes in that conditions which result in the appearance of more material in the pigmented particles which are separated by centrifugation in a gradient (peak B, Figure 1) also give rise to more material in the peak containing the ribosomes (peak C, Figure 1). It seems fair to suggest that if the 160 S particles are an artifact then so are the ribosomes and, conversely, if the latter are real structures so are the former.

Use of Moving Boundary Sedimentation. The technique of moving boundary sedimentation in the preparative ultracentrifuge, first investigated thoroughly by Trautman and Breese (1959), probably has not been used as widely as it deserves, having been overshadowed by the methods for determining s values by zone centrifugation in sucrose gradients (de Duve *et al.*, 1959). However it has certain advantages which are worth enumerating. Sedimentation coefficients can be determined in sucrose gradients only if the density of the equivalent hydrodynamic sphere is known. It is usual to assume a value of about 1.40 g/cm^3 for the density when the material to be analyzed is a protein, and as Martin and Ames (1961) have shown this leads to errors of less than 3% if the true density lies between 1.35 and 1.45 g/cm^3. However, when the density lies outside these limits the s value calculated in this way may be grossly in error, and indeed if the method had been applied to the pigmented particles of *Rps. spheroides* a quite erroneous picture would have been obtained. By inserting the correct value for the density in the equations of de Duve *et al.* and carrying out a numerical integration it would have been possible to calculate an s value from a sucrose gradient; the moving boundary method used here gave a value of s without the necessity of knowing the density, and the density was itself calculated from a series of s values. A further practical advantage of the moving boundary method used here is that as the salt gradients were employed only to stabilize the boundaries against convection it did not matter that they were not constructed with great accuracy; consequently, a relatively crude device could be used for this purpose in place of the more elaborate apparatus used by de Duve *et al.* (1959) or Martin and Ames (1961). Finally, the 55 S particles which contain a small fraction of the photosynthetic pigments were detected as a boundary in these experiments, but their existence could only be demonstrated by zone centrifugation in occasional extracts which happened to have a higher content of these particles than normal.

Appendix

Calculation of the Mean and Variances of μ. The initial (x_0) and final (x) coordinates of a particle sedimenting in a reciprocal density gradient with constant velocity $\mu\omega^2$ are related by

$$x = x_0 + \mu\omega^2 t \tag{1A}$$

and the concentration c_p of particles in the plateau region stays equal to the initial concentration c_0 (Gibson, 1965b). If $p(\mu)$ is the probability density of the distribution of μ, then any particle whose coordinate is x at time t must have a value of μ which satisfies eq 1A, with x_0 greater than or equal to the coordinate x_1 of the meniscus. Thus the concentration at the point x at time t is

$$c(x, t) = c_0 \int_0^{(1/\omega^2 t)(x-x_1)} p(\mu)\mathrm{d}\mu \tag{2A}$$

From this equation

$$\frac{\partial c}{\partial t} = \frac{c_0}{\omega^2 t}\, p\left\{\frac{1}{\omega^2 t}(x - x_1)\right\} \tag{3A}$$

Assume that the coordinate of the bottom of the gradient (x_2) is so large that no particles originally at the meniscus reach it in the time t; this means that $p(\mu) = 0$ if $\mu > (1/\omega^2 t)(x_2 - x_1)$. Then x_2 is in the plateau region and $c(x_2) = c_p = c_0$. Using this relation and eq 3A the rth moment of the distribution of μ is found to be

$$\begin{aligned}
\int_0^\infty \mu^r p(\mu)\mathrm{d}\mu &= \int_0^{(1/\omega^2 t)(x_2-x_1)} \mu^r p(\mu)\mathrm{d}\mu \\
&= \frac{1}{(\omega^2 t)^{r+1}} \int_{x_1}^{x_2} (x - x_1)^r p\left\{\frac{1}{\omega^2 t}(x - x_1)\right\}\mathrm{d}x \\
&= \frac{1}{c_0(\omega^2 t)^r} \int_{x_1}^{x_2} (x - x_1)^r \frac{\partial c}{\partial x}\,\mathrm{d}x \\
&= \frac{1}{(\omega^2 t)^r}\left\{(x_2 - x_1)^r - \frac{r}{c_p}\int_{x_1}^{x_2} (x - x_1)^{r-1}\, c(x)\mathrm{d}x\right\}
\end{aligned} \tag{4A}$$

Equations 6 and 7 in the text are obtained by putting $r = 1$ and $r = 2$ in eq 4A.

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References

Aldrich, K. M., and Sword, C. P. (1964), *J. Bacteriol. 87*, 690.

Bahr, G. F., and Zeitler, E. (1962), *J. Cell Biol. 15*, 489.

Bergeron, J. A. (1958), *Brookhaven Symp. Biol. 11*, 118.

Bergeron, J. A., and Fuller, R. C. (1961), *in* Biological Structure and Function, Vol. II, Goodwin, T. W., and Lindberg, O., ed., New York, Academic, p. 307.

Boatman, E. S. (1964), *J. Cell Biol. 20*, 297.

Bock, R. M., and Ling, N. (1954), *Anal. Chem. 26*, 1543.

Britten, R. J., and Roberts, R. B. (1960), *Science 131*, 32.

Bull, M. J., and Lascelles, J. (1963), *Biochem. J. 87*, 15.

Cohen-Bazire, G. (1963), *in* Bacterial Photosynthesis, Gest, H., San Pietro, A., and Vernon, L. P., ed., Yellow Springs, Ohio, Antioch Press, p. 89.

Cohen-Bazire, G., and Kunisawa, R. (1960), *Proc. Natl. Acad. Sci. U. S. 46*, 1543.

Cohen-Bazire, G., and Kunisawa, R. (1963), *J. Cell Biol. 16*, 401.

Cohen-Bazire, G., Sistrom, W. R., and Stanier, R. Y. (1957), *J. Cellular Comp. Physiol. 49*, 25.

de Duve, C., Berthet, J., and Beaufay, H. (1959), *Progr. Biophys. 9*, 325.

French, C. S., and Milner, H. W. (1955), *Methods Enzymol. 1*, 64.

Frenkel, A. W. (1954), *J. Am. Chem. Soc. 76*, 5568.

Frenkel, A. W., and Hickman, D. D. (1959), *J. Biophys. Biochem. Cytol. 6*, 285.

Gibson, K. D. (1964a), Abstracts of the 6th International Congress of Biochemistry, New York, p. 777.

Gibson, K. D. (1964b), *Biochem. J., 93*, 21P.

Gibson, K. D. (1965a), *Biochemistry 4*, 2042 (this issue; following paper).

Gibson, K. D. (1965b), *J. Phys. Chem. 69*, 1820.

Gibson, K. D. (1965c), *J. Bacteriol.* (in press).

Gibson, K. D., Neuberger, A., and Tait, G. H., (1962), *Biochem. J. 83*, 539.

Hickman, D. D., and Frenkel, A. W. (1959), *J. Biophys. Biochem. Cytol. 6*, 277.

Karunairatnam, M. C., Spizizen, J., and Gest, H. (1958), *Biochim. Biophys. Acta 29*, 649.

Lascelles, J. (1959), *Biochem. J. 72*, 508.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

Martin, R. G., and Ames, B. N. (1961), *J. Biol. Chem. 236*, 1372.

McQuillen, K. (1960), *in* The Bacteria, Vol. I, Gunsalus, I. C., and Stanier, R. Y., ed., New York, Academic, p. 249.

Newton, J. W., and Newton, G. A. (1957), *Arch. Biochem. Biophys. 71*, 250.

Palade, G. E., and Siekevitz, P. (1956), *J. Biophys. Biochem. Cytol. 2*, 671.

Schachman, H. K., Pardee, A. B., and Stanier, R. Y. (1952), *Arch. Biochem. Biophys. 38*, 245.

Thomson, J. F., and Klipfel, F. J. (1957), *Arch. Biochem. Biophys. 70*, 224.

Trautman, R., and Breese, S. (1959), *J. Phys. Chem. 63*, 1592.

Tuttle, A. I., and Gest, H. (1959), *Proc. Natl. Acad. Sci. U. S. 45*, 1261.

Vatter, A. E., and Wolfe, R. S. (1958), *J. Bacteriol. 75*, 480.

Vernon, L. P., and Ash, O. K. (1959), *J. Biol. Chem. 234*, 1878, 1883.

Warburg, O., and Christian, W. (1941), *Biochem. Z. 310*, 384.

Worden, P. B., and Sistrom, W. R. (1964), *J. Cell Biol. 23*, 135.