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MAGNETIC FIELD INDUCED ORIENTATION OF PHOTOSYNTHETIC SYSTEMS

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SUMMARY

1. The fluorescence of aqueous suspensions of *Chlorella, Scenedesmus, Euglena* and spinach chloroplasts is preferentially polarized in a plane perpendicular to an external magnetic field of 10 kG or more. The ratio of the fluorescence intensity viewed perpendicular to the field to the intensity viewed parallel to the field varies from 1.03 to 1.57.

2. The suspensions also exhibit dichroism and anisotropic wavelength-dependent light scattering effects which are induced by the magnetic field. The dichroic maximum may nearly coincide with the absorption maximum of the bulk pigments and in some cases is shifted to the red by selective light scattering.

3. It is concluded that the dichroism and fluorescence polarization are due to a preferred orientation of the chlorophyll porphyrin rings, and the plane of the lamellae, perpendicular to the field.

4. If it is assumed that the magnetic field does not reorient individual chlorophyll molecules, then these results imply that chlorophyll *in vivo* possesses a higher degree of orientation than previously thought.

5. It is shown for *Chlorella* that the magnetic field induces a reorientation of the entire cell.

6. The physical basis of these effects can be adequately explained in terms of an anisotropy in the diamagnetic susceptibility of the cell components.

7. Magnetic field induced orientation can be used to study the optical properties of a large number of suspended oriented cells *in vivo*.

INTRODUCTION

The degree of orientation of chlorophyll *in vivo* has been the subject of many investigations. In recent years Olson and co-workers[1–5] have studied the wavelength

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dependence of the dichroism and fluorescence polarization of single chloroplasts. A dichroism was observed at wavelengths longer than 690 nm, but not in the region of the main absorption band which is situated at $\approx$ 675 nm. The fluorescence in the wavelength region of the emission maximum near 685 nm was found to be depolarized; however, some polarization was observed at wavelengths above 690 nm. Sauer and Calvin[6] have observed electric field induced dichroism in spinach chloroplast fragments (quantasomes); the maximum in the dichroic ratio was found at 695, about 17 nm to the long wavelength side of the absorption maximum. It was concluded from these studies that the main bulk of chlorophyll molecules have a low degree of orientation, but that a longer wavelength absorbing form of chlorophyll is highly oriented relative to the planes of the chloroplast lamellae. The conclusion that the main bulk of pigments is largely unoriented was also reached by Goedheer[7].

Further evidence that the bulk pigments have a low degree of mutual orientation was obtained from fluorescence polarization studies. The degree of polarization P is defined by

$$P = \frac{F_{\parallel} - F_{\perp}}{F_{\parallel} + F_{\perp}} \tag{1}$$

The fluorescence is excited with polarized light and is viewed at a 90° angle to the exciting light beam through an analyzer. $F_{\parallel}$ and $F_{\perp}$ are the fluorescence intensities measured with the electric vector direction of the analyzer oriented parallel and perpendicular respectively to the electric vector direction of the polarizer. For *Chlorella*, P values in the range of 0.01–0.06 have been reported[8–10], although higher values were quoted by Lavorel[11]. This low degree of polarization *in vivo* is generally taken as evidence that extensive energy transfer takes place between the antenna pigment molecules. The polarization "memory" is destroyed by the low degree of orientation of the pigment molecules and the large number of excitation transfers.

We have found that an external magnetic field can induce a considerable fluorescence polarization and dichroism of chlorophyll *in vivo*. These effects depend on the direction of excitation and direction of viewing of the fluorescence with respect to the magnetic field. A preliminary report describing these effects in *Chlorella* has been published[12]. In this paper we show that changes in the fluorescence polarization can be induced in other organisms such as *Euglena, Chlamydomonas, Scenedesmus* and spinach chloroplasts. In the presence of the magnetic field the chlorophyll molecules are oriented in such a way that the transition moment of the lowest electronic transition (the "red" chlorophyll band) tends to lie in a plane perpendicular to the field. The entire *Chlorella* cells are shown to reorient themselves in the field, and the results are discussed in terms of possible orientation of pigments *in vivo*. The magnetic effects are consistent with a mechanism which assumes an anisotropy in the diamagnetism of the cell components.

METHODS

The *Chlorella pyrenoidosa, Chlorella vulgaris* and *Scenedesmus obliquus* were grown in an inorganic medium as described previously[12]. The *Euglena gracilis* was

grown in the medium of Greenblatt and Schiff[13] (Medium A). The spinach chloroplasts were prepared according to the method of Stiehl and Witt[14].

The high field fluorescence experiments were performed in a Bitter Solenoid magnet at the National Magnet Laboratory. Homogeneous and steady fields up to 100 kG could be generated in the 2-inch diameter bore. The apparatus was the same as that previously described[12], except that it was modified to accept sheet polarizers (Polaroid Corporation, type HNB). Some of the measurements with *Chlorella pyrenoidosa* were carried out at 10.5 kG (near saturation) with an iron core magnet.

The fluorescence in all experiments was viewed with a cooled RCA 7102 photomultiplier fitted with a Corning C.S. 2-64 filter (short wavelength 640-nm cut-off filter). Thus the entire emission was viewed, with the dominant contribution from the $\approx$ 685-nm chlorophyll *a* fluorescence band. The photocurrent was fed into picoammeters which were hooked up to X–Y or strip-chart recorders.

The light for the transmittance experiments was isolated from a 750-W tungsten lamp by a monochromator whose wavelength drum was equipped with a motor drive. The light was collimated (3–4°) by a multiple slit assembly. The algal suspensions were placed in a 1.5-mm optical path cuvette. The light transmitted by the suspensions was viewed with a fiber optic placed directly behind the cuvette (estimated collecting angle $\approx 27°$) which fed the light to an RCA 7164R photomultiplier. The photomultiplier current was amplified and fed into an Intertechnique DIDAC 800 digital analyzer. A photodiode was used to monitor the intensity of the lamp; this signal was fed into another section of the DIDAC memory. The memory advance of the analyzer was controlled by the wavelength drum of the monochromator. The system was designed so that a complete scan from 610 to 735 nm could be accomplished in 16 sec, thus minimizing the problem of the settling of the algae, which could give rise to trivial changes in the transmittance.

The cell rotation (*Chlorella* and *Scenedesmus*, although results for *Chlorella* only will be reported here) was established by changing the external viscosity. A water-soluble synthetic polymer Ficoll (Pharmacia, Uppsala, Sweden, lot No. 6071, Number average molecular weight $\approx 4 \cdot 10^5$) was added to the algal suspensions and the relaxation of the magnetic field induced changes in the fluorescence was monitored as a function of time after the magnetic field had been removed.

RESULTS

Fluorescence

The magnetic field dependence of the fluorescence viewed in a direction perpendicular to that of the magnetic field $\boldsymbol{H}$ is shown in Fig. 1. The fluorescence was viewed along the z axis with the electric vector directions of the analyzer oriented either along the y or x axis (labeled a_y and a_x, respectively). The exciting light beam was oriented along the y axis, perpendicular to both the magnetic field and fluorescence viewing directions; the orientation of the polarizer (p_x in Fig. 1) was parallel to $\boldsymbol{H}$, *i.e.* along the x axis. In all cases the fluorescence decreased when the analyzer was oriented parallel (a_x) to $\boldsymbol{H}$ and increased when it was oriented perpendicular (a_y) to the field. No magnetic field effect was observed with the blue-green alga *Phormidium* or with chlorophyll *a* dissolved in benzene. All the organisms, except *Euglena*, exhibit a field saturation at magnetic fields below 25 kG. The behavior of

Euglena was markedly different in that the magnetic field induced changes in the fluorescence did not saturate even at fields as high as 100 kG. *Euglena* is different from the other organisms investigated here in that it is an algal flagellate and exhibits phototactic behavior.

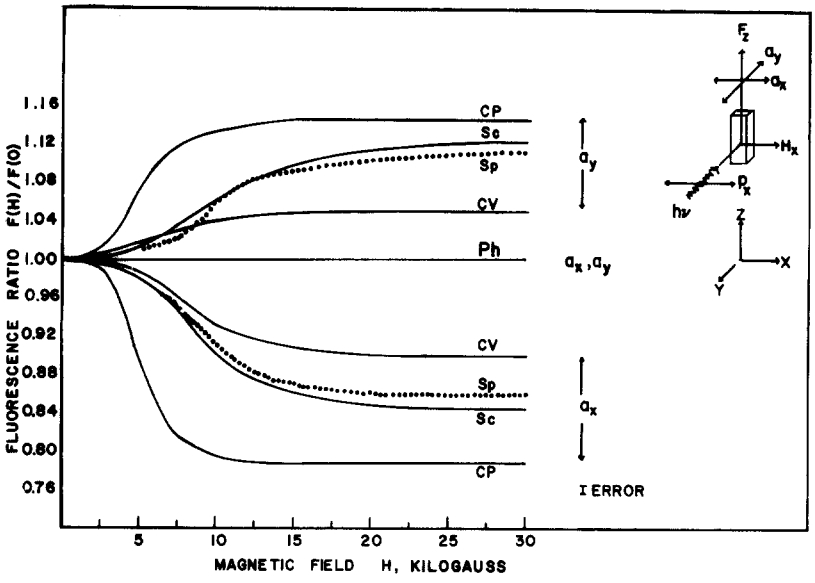

Fig. 1. Polarized fluorescence of chlorophyll *a in vivo* as a function of magnetic field strength $F(\boldsymbol{H})$, fluorescence intensity in the magnetic field $\boldsymbol{H}$; $F(0)$, zero field fluorescence; F_z, fluorescence viewing direction; a_x, a_y, analyzer orientation either parallel or perpendicular to $\boldsymbol{H}$; $h\nu$, direction of exciting light beam; P_x, polarizer orientation. Wavelength of exciting light: predominantly the 406- and 436-nm lines of a 100-W mercury lamp, intensity $\approx 5 \cdot 10^3$ ergs·cm^{-2}·sec^{-1}. CP, *Chlorella pyrenoidosa* (No. 395), Sc, *Scenedesmus obliquus*, Sp, fresh spinach chloroplasts, CV, *Chlorella vulgaris*, Ph, *Phormidium luridum*.

We denote the fluorescence intensity viewed with the analyzer oriented perpendicular to the magnetic field by $F(\perp\boldsymbol{H})$ and the intensity viewed with the analyzer oriented parallel to $\boldsymbol{H}$ by $F(\parallel\boldsymbol{H})$. Since $F(\perp\boldsymbol{H})$ is greater than $F(\parallel\boldsymbol{H})$ in all cases, we conclude that the magnetic field tends to orient the chlorophyll molecules in such a way that the total projection of the transition moment vectors is greater perpendicular to $\boldsymbol{H}$ than parallel to $\boldsymbol{H}$. The fluorescence polarization ratio $F(\perp\boldsymbol{H})/F(\parallel\boldsymbol{H})$ varies with the different organisms as well as with different species of *Chlorella*. Considerable variations are also encountered with different cultures of the same strain of *Chlorella pyrenoidosa*, which we have investigated more extensively than the other organisms. With spinach chloroplasts the fluorescence polarization ratio also depends on the condition of the chloroplasts. Freshly prepared spinach chloroplasts exhibited a higher effect than chloroplasts which had been frozen (for storage purposes). The freezing probably tends to disrupt the lamellar structure and these results suggest that the magnetic field induced fluorescence changes depend on the internal structure and organization of the lamellae.

Results on the fluorescence polarization ratio are summarized in Table I.

The highest magnetic field effects were observed with *Chlorella pyrenoidosa*,

TABLE I

VALUES OF THE MAGNETIC FIELD INDUCED FLUORESCENCE POLARIZATION RATIO $F(\perp H)/F(\parallel H)$ AT MAGNETIC FIELD STRENGTHS CORRESPONDING TO SATURATION

Organism	*Culture No.* *	$F(\perp H)/F(\parallel H)$ *ratio*
Fresh spinach chloroplasts	—	1.29
Pre-frozen spinach chloroplasts	—	1.03–1.08
Chlorella pyrenoidosa **	395	1.30–1.57
Chlorella pyrenoidosa	252	1.10
Chlorella vulgaris	397	1.17
Scenedesmus obliquus	72	1.33
Chlamydomonas reinhardii ***	—	1.07
Phormidium luridum†	—	1.00
Euglena gracilis	369	1.1–1.4††

* Indiana University Culture Collection Number.
** Chick Emerson strain from Govindjee's laboratory, University of Illinois.
*** Sample from the laboratory of R. P. Levine, Harvard University.
† Sample from the laboratory of D. Mauzerall, Rockefeller University.
†† Value at 100 kG, not saturated with respect to magnetic field; depends strongly on orientations of exciting light beam and polarizer with respect to magnetic field.

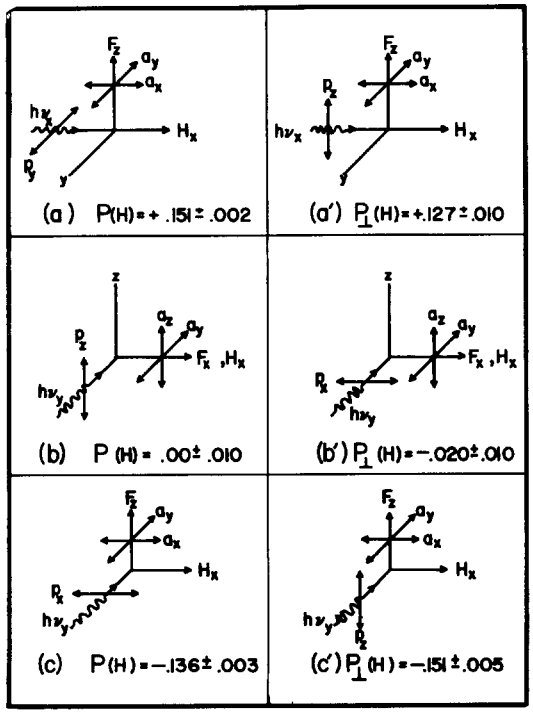

Fig. 2. Degree of polarization P of the fluorescence of a *Chlorella pyrenoidosa* (No. 395) suspension at 10.5 kG. F, fluorescence viewing direction; H, magnetic field direction; a, orientation of analyzers; p, orientation of polarizer. a', b', c' configurations are the same as in a, b and c, respectively, except that electric vectors of all polaroid sheets are orthogonal (see text for definition of $P_\perp$). Wavelength of excitation: 620 ± 10 nm, intensity ≈100 ergs·cm^{-2}·sec^{-1}.

Strain 395. Another strain, Indiana Collection No. 252, gave consistently lower effects. Most of our work was done on *Chlorella pyrenoidosa* (No. 395).

Degree of fluorescence polarization of Chlorella suspensions

The value of the degree of polarization measured in the magnetic field, $P(\boldsymbol{H})$, depends on the mode of excitation and viewing direction, and its absolute value is usually larger than the corresponding value in zero-field, $P(0)$. Results for a given sample of *Chlorella* are summarized in Fig. 2. The $P(\boldsymbol{H})$ values are given under the diagrams which show the relative orientations of the light beam ($h\nu$), polarizers (p), analyzers (a), magnetic field and fluorescence viewing directions (F). In zero field the degree of polarization is $P(0) = +0.015 \pm 0.010$, independent within experimental error of the measurement configuration. The error ranges associated with these P values are a reflection of the precision, but not the accuracy of the measurements (*i.e.* systematic errors are not taken into account). For example, the exciting and viewing light beams were not collimated because a maximum signal/noise ratio was desired. Weber[15] has shown that this condition tends to give lower values of P.

The degree of polarization P in Fig. 2 is defined as follows:

$$\frac{F(a_y) - F(a_x)}{F(a_y) + F(a_x)} \quad \text{in Figs 2a and 2a'} \tag{2}$$

$$\frac{F(a_z) - F(a_y)}{F(a_z) + F(a_y)} \quad \text{in Figs 2b and 2b'} \tag{3}$$

$$\frac{F(a_x) - F(a_y)}{F(a_x) + F(a_y)} \quad \text{in Figs 2c and 2c'} \tag{4}$$

where $F(a_y)$, $F(a_x)$ and $F(a_z)$ are the fluorescence intensities measured with the analyzer oriented as shown in Fig 2. The degree of polarization in the magnetic field, $P(\boldsymbol{H})$ given in Figs 2a, 2b, and 2c is properly defined according to Eqn 1 (*i.e.* the orientation of one of the analyzers is parallel to that of the polarizers). For $P\perp(\boldsymbol{H})$ in Figs 2a', 2b', 2c', however, the polarizer is orthogonal to both analyzer directions.

If the orientation of the emitting oscillators is random, $P\perp(\boldsymbol{H})$ should be zero in all cases since both analyzer directions are perpendicular to that of the polarizer. We observe, however that in all cases $P(\boldsymbol{H}) \approx P\perp(\boldsymbol{H})$; in fact, similar P values are obtained even if the exciting light is unpolarized. These results show conclusively that the fluorescence polarization shown in Fig. 1 is primarily due to a preferred orientation of the emitting molecules with respect to the magnetic field, and not due to an enhanced polarization "memory". The approximate equivalence of $P(\boldsymbol{H})$ and $P\perp(\boldsymbol{H})$ can be explained by taking into account energy transfer from the absorbing molecules to a group of oriented emitting oscillators. In Fig. 2c, the absorbing oscillators are oriented perpendicular (along x) to the preferred orientation of the emitting oscillators which is the direction perpendicular to $\boldsymbol{H}$; the degree of polarization is therefore negative. In Fig. 2a, the polarizer is oriented parallel to the preferred orientation of the oscillators which is in the plane perpendicular to the magnetic field (the yz plane) and P is positive. The orientation of the oscillators in this plane

is random, as is clearly shown in Fig. 2b—in this case $P(\boldsymbol{H}) \approx 0$, since both analyzer directions are oriented perpendicular to $\boldsymbol{H}$.

If the magnetic field has a tendency to increase the degree of mutual orientation of chlorophyll molecules, it is conceivable that the degree of polarization may increase as a result. However, the quantity $|P(\boldsymbol{H}) - P\perp(\boldsymbol{H})| \approx P(0)$ within experimental error; we therefore conclude that any mutual alignment of pigments, if it occurs at all, is not sufficient to reduce the depolarization by energy transfer.

Magnetic field induced dichroism of chlorella suspensions

The fluorescence polarization results suggest that a magnetic field induced dichroism in the main absorption band at 675–678 nm should also occur. Such a magnetic field induced dichroism is indeed observed as is shown in Fig. 3. When the light beam is oriented parallel to the field ($h\nu \| \boldsymbol{H}$, Fig. 3a), there is an overall increase of about +7% in the transmitted light, on which there is superimposed a dip at 677 nm which coincides with the absorption maximum. The overall +7% "base line" in Fig. 3a is due to a field induced increase in the light scattering efficiency in the direction parallel to the magnetic field, which is also the direction along which the transmitted light is viewed. The dip in the transmitted light corresponds to an

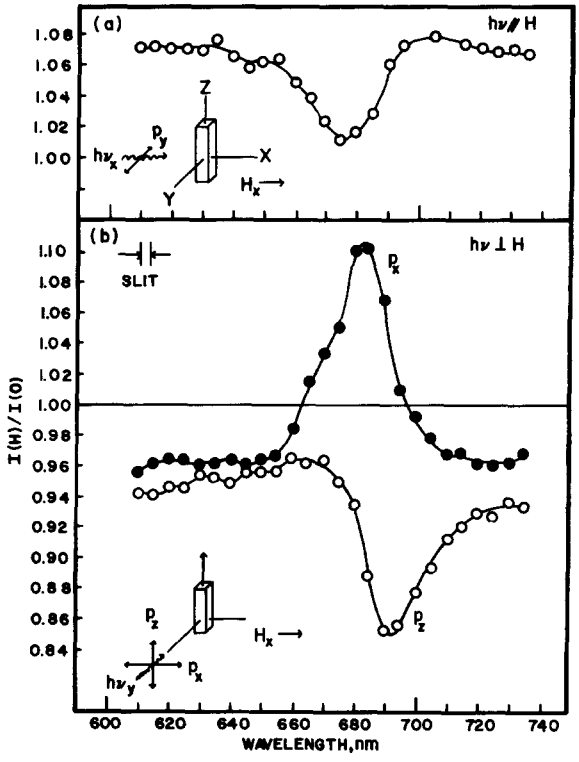

Fig. 3. Magnetic field induced changes in the transmittance of *Chlorella pyrenoidosa* (No. 395) suspensions at 10.5 kG. (a) Direction of light beam is parallel to the field ($h\nu \| \boldsymbol{H}$). (b) Direction of light beam is perpendicular to $\boldsymbol{H}$ ($h\nu \perp \boldsymbol{H}$); p, orientation of electric vector of polarizer. $I(\boldsymbol{H})$ and $I(0)$ are the intensities of the transmitted light in the magnetic field and in zero field respectively. The average number of cells was $22 \cdot 10^6$ cm^{-3}.

increase in the absorption of the algal suspensions. The theory of the flattening effect[16] on the absorption spectrum of particles suspended in a medium suggests that the change in absorption should appear the largest at the absorption peak of the suspension, as is observed experimentally (Fig. 3). Thus a peak or dip in the $I(\boldsymbol{H})/I(0)$ curve coincides with the absorption maximum. Another noteworthy fact is that in the $h\nu \| \boldsymbol{H}$ orientation, the $I(\boldsymbol{H})/I(0)$ curve shown is independent, within experimental error, of the orientation of the polarizer electric vector (p) of the incident light. This is consistent with the observation that the fluorescence is isotropic when viewed in a direction parallel to $\boldsymbol{H}$.

In the $h\nu \perp \boldsymbol{H}$ orientation, $I(\boldsymbol{H})/I(0)$ is a function of the orientation of the polarizer p. Thus, when p is oriented perpendicular to the magnetic field (z in Fig. 3b), there is a decrease in transmittance and therefore an increase in the absorbance. When p is oriented parallel to $\boldsymbol{H}$ (p_x in Fig. 3b), there is a decrease in the absorption. Unlike in Fig. 3a, the peaks in Fig. 3b are displaced toward the red with respect to the absorption maximum at 675–678 nm. It is shown elsewhere[17], that this is due to a magnetic field induced decrease in the selective light scattering[18] in the direction perpendicular to $\boldsymbol{H}$.

The absorption increases when the electric vector of the light is at a right angle to the magnetic field and decreases when it is parallel to $\boldsymbol{H}$. This indicates that the absorbing oscillators tend to orient at 90° to $\boldsymbol{H}$. Since the 675-nm absorption band has the same polarization as the fluorescence, the magnetic field induced dichroism is in accord with the fluorescence polarization studies. Furthermore, the magnetic field induced changes in the transmittance of *Chlorella* suspensions exhibit the same field strength dependence as that of the fluorescence shown in Fig. 1. This is another indication that these two effects are related. However, quantitative comparisons[12] between the absolute changes in the absorbance of the suspensions and changes in the fluorescence cannot be made easily. The magnetic field induced changes in the absorbance of a cell suspension increases with increasing cell density[17], whereas the changes in the fluorescence intensity reflect changes in a single cell (if the cell density is sufficiently low to minimize reabsorption of fluorescence). Furthermore, such comparisons are complicated by the anisotropic and wavelength dependent changes in the light scattering efficiency[17].

Viscosity effect on relaxation times

It was shown previously[12] that when the magnetic field is quickly reduced to zero the magnetic field induced changes in the fluorescence persisted for a long time. This relaxation time is markedly increased by increasing the viscosity of the aqueous suspensions with a high molecular weight polymer (Ficoll). We assume that the polymer does not penetrate through the cell walls. Microscope examination of the *Chlorella* suspended in Ficoll solution indicated that their size did not change in the Ficoll solution and that the cells were intact. The viscosity effect was found to be reversible—the Ficoll suspensions were centrifuged, washed with and resuspended in Ficoll-free medium. The relaxation time reverted to its original value. Upon standing in Ficoll suspensions for prolonged periods (several hours), the relaxation time increased gradually. Our measurements were therefore made within ten minutes of suspending the *Chlorella* cells in the viscous solutions. Three typical measurements are shown in Fig. 4. The fluorescence was viewed with the analyzer oriented per-

pendicular to the field. Thus the measured fluorescence intensity while the magnetic field was on, $F(\boldsymbol{H})$, was greater than the equilibrated zero-field value, $F(o)$. The magnetic field was turned off and required 4 s to reach zero and the fluorescence decay in this time period is indicated by vertical bars at $t = o$ in Fig. 4. The field

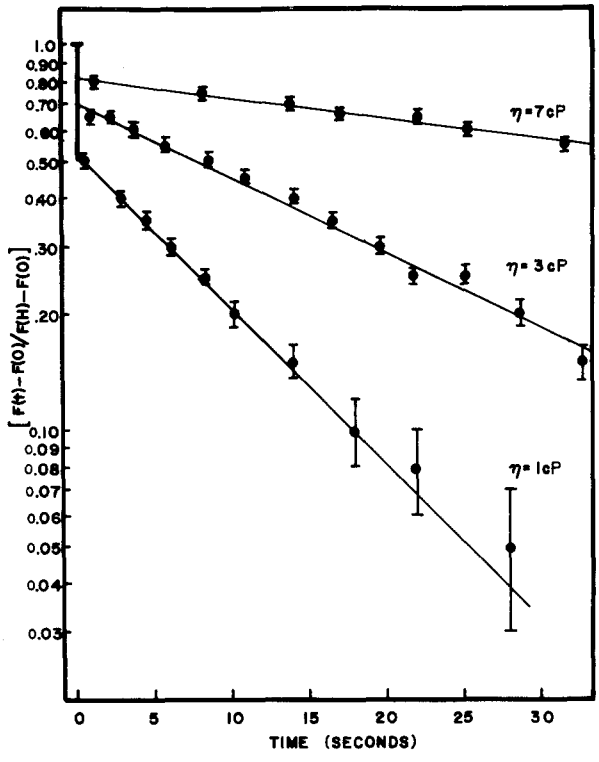

Fig. 4. Viscosity dependence of the relaxation of the magnetic field induced changes in the fluorescence. $F(\boldsymbol{H})$, fluorescence at $\boldsymbol{H} = 10.5$ kG; $F(o)$, fluorescence in zero field. The analyzer was oriented perpendicular to $\boldsymbol{H}$. $F(t)$, decay of fluorescence from $F(\boldsymbol{H}) \rightarrow F(o)$ after field had been removed. Drop in fluorescence during the four second magnetic field shut-off time indicated by vertical bar at $t = o$.

strength was monitored with a Hall effect probe and the strip-chart recorder was started as soon as the field reached zero. The decay of the fluorescence from $F(\boldsymbol{H})$ to $F(o)$, which is labeled $F(t)$, was thus monitored as a function of time. The decay of the fluorescence in Fig. 4 is expressed as the time dependent fractional decay of the magnetic field induced change. Thus, at the moment that the field is turned off, $F(t = o) = F(\boldsymbol{H})$ and after a sufficiently long time $F(t) \rightarrow F(o)$. The magnetic field itself takes about four seconds to decay to zero. During this time, the magnetic field induced change in the fluorescence drops by 50% at 1 cP (centipoise), and 30 and 19% at 3 and 7 cP, respectively. The decay for $t > o$ is exponential as can be seen in Fig. 4. The time required for the effect to decay by an additional fraction 1/e (e = 2.718) was 11, 23 and 60 s at 1, 3 and 7 cP, respectively.

DISCUSSION

The viscosity dependence

The conclusion about the cell reorientation in the magnetic field can be further strengthened if it can be shown that the cell diameter calculated from the exponential relaxation time shown in Fig. 4 agrees approximately with the directly observed diameter.

As soon as the magnetic field is removed, the cells are free to undergo Brownian motion which randomizes the orientation of the cells. If we make the simplifying assumption that the *Chlorella* cells are rigid homogeneous spheres, the relaxation time can be calculated by solving the rotational diffusion equation. We define an observation axis Z along which the polarized fluorescence is viewed, and some arbitrary fixed radius vector r within the spheres which makes an angle of $\omega = 0$ degree with Z at time $t = 0$. We further assume that the contribution to the measured fluorescence from each particle is proportional to the average value of $\cos^2\omega(t)$ which decreases with time after the orienting magnetic field is removed at $t = 0$, where $\omega(t)$ is the angle between r and Z. The average projection of $\cos^2\omega(t)$ onto Z as a function of time is then given by a solution of the rotational diffusion equation[19] which gives:

$$\langle \cos^2 \omega(t) \rangle = \tfrac{1}{3} + \tfrac{2}{3} e^{-6Rt} \tag{5}$$

where $R = \boldsymbol{k}T/6\eta V$ and the relaxation time τ is defined as

$$\tau = \frac{1}{6R} = \frac{\eta V}{\boldsymbol{k}T} \tag{6}$$

In these expressions V is the volume of the sphere, η the viscosity of the solution in which the sphere is suspended, $\boldsymbol{k}$ is Boltzmann's constant and T is the absolute temperature. Eqn 5 merely expresses the fact that when all cells are aligned $\langle \cos^2\omega(t) \rangle = 1$ (at $t = 0$) and is $^1/_3$ when the cells are randomized at $t = \infty$.

Using the data of Fig. 4 and Eqn 6, we calculate the following cell diameters from the slopes of the decay curves: 4.3 μm at 1 cP, 4.0 μm at 3 cP and 4.1 μm at 7 cP. A series of six other measurements on another culture of *Chlorella pyrenoidosa* in the viscosity range of 1–10 cP gave values of 3.7 $\pm$ 0.1 μm for the diameter. Visual examination of the *Chlorella* cells under a microscope showed that the average size was about 3 μm with a distribution ranging from 1–4 μm. Since *Chlorella* cells are neither rigid nor homogeneous spheres, the agreement between the calculated and directly observed diameters is considered to be very good.

The dependence on the magnetic field strength

The most probable physical origin of the effects described here is an anisotropy in the diamagnetic susceptibility of some of the cell components. Hong *et al.*[20] have indicated quantitatively that this is a reasonable mechanism to explain the magnetic field induced orientation of retinal rods. It is shown below that the qualitative aspects of the dependence of the effects on magnetic field strength, such as shown in Fig. 1, are in accord with this hypothesis. What has to be explained theoretically is the relatively small effect at low magnetic field strengths, a sharp superlinear increase at intermediate field strengths and, finally, a saturation at higher fields.

The magnetic potential energy of a magnetically anisotropic sample of effective volume V in a homogeneous magnetic field may be written as

$$U = -\tfrac{1}{2}V\boldsymbol{H}^2(\chi_1 l^2 + \chi_2 m^2 + \chi_3 n^2) \tag{7}$$

The reference axes were chosen in such a way that the susceptibility tensor is diagonal with volume susceptibilities χ_1, χ_2 and χ_3 along the principal axes. The field $\boldsymbol{H}$ makes an angle with this orthogonal set of axes whose direction cosines are l, m, and n. In order to simplify the equations to follow, we make the arbitrary assumption that $\chi_2 \approx \chi_3$; this assumption, however, does not change the basic forms of the equations to follow and, therefore, the basic conclusions. With this assumption, the field $\boldsymbol{H}$ can therefore always be considered as being oriented in a plane defined by the directions along which χ_1 and χ_2 are defined since χ_2 has axial symmetry about the axis defining χ_1. The magnetic energy becomes

$$U = -\tfrac{1}{2}V\boldsymbol{H}^2(\Delta\chi\cos^2\theta + \chi_2) \tag{8}$$

where

$$\Delta\chi = \chi_1 - \chi_2$$

The probability of finding the particle whose magnetic axis (along which the volume susceptibility has the value χ_1) makes an angle θ with the field $\boldsymbol{H}$ in the solid angle $2\pi \sin\theta\, \mathrm{d}\theta$ is proportional to

$$\mathrm{e}^{-U/kT} \propto \mathrm{e}^{\alpha^2\cos^2\theta} \tag{9}$$

where

$$\alpha^2 = \tfrac{1}{2}\Delta\chi V\boldsymbol{H}^2/kT \tag{10}$$

We assume that the direction of the fluorescence emitting dipole has a specified direction with respect to the magnetic axis. If we evaluate the average projection $\langle\cos^2\theta\rangle$ of the magnetic axis onto the direction of the field $\boldsymbol{H}$, we should be able to evaluate the change in the fluorescence intensity viewed along the direction defining $\boldsymbol{H}$, (or perpendicular to $\boldsymbol{H}$), as a function of magnetic field strength. This is the case because the fluorescence emitting dipole has a fixed orientation with respect to the magnetic axis, and the latter tends to align itself with $\boldsymbol{H}$ as the field increases.

To illustrate the dependence of the fluorescence on the magnetic field strength, we shall consider a particular simple case. We consider the direction of the transition moment vector to be parallel to the magnetic axis because in that case the fluorescence intensity will be proportional to $\langle\cos^2\theta\rangle$. (If the transition moment is perpendicular to the magnetic axis, then the fluorescence viewed along $\boldsymbol{H}$ would be proportional to $\tfrac{1}{2}(1-\langle\cos^2\theta\rangle)$. The average projection $\langle\cos^2\theta\rangle$ onto the direction of $\boldsymbol{H}$ is

$$\langle\cos^2\theta\rangle = \frac{\int_0^\pi \cos^2\theta\, \mathrm{e}^{\alpha^2\cos^2\theta}\sin\theta\,\mathrm{d}\theta}{\int_0^\pi \mathrm{e}^{\alpha^2\cos^2\theta}\sin\theta\,\mathrm{d}\theta} \tag{11}$$

We make the substitution $y = \cos\theta$ and obtain

$$\langle\cos^2\theta\rangle = \frac{\int_0^1 y^2\,\mathrm{e}^{\alpha^2y^2}\,\mathrm{d}y}{\int_0^1 \mathrm{e}^{\alpha^2y^2}\,\mathrm{d}y} \tag{12}$$

If we make still another substitution, $p^2 = \alpha^2 y^2$ it is possible to integrate the numerator in Eqn 12 by parts:

$$\langle \cos^2 \theta \rangle = \frac{\int_0^\alpha p^2 e^{p^2} dp}{\alpha^2 \int_0^\alpha e^{p^2} dp} = \tag{13}$$

$$= \frac{e^{\alpha^2}}{2\alpha \int_0^\alpha e^{p^2} dp} - \frac{1}{2\alpha^2} \tag{14}$$

In the limit when $\alpha \ll 1$, *i.e.* at very low magnetic fields, the exponentials in Eqn 14 can be expanded in a power series. After integrating and simplifying, the following result is obtained for $\alpha^2 \ll 1$:

$$\langle \cos^2 \theta \rangle = \frac{1}{3} + \frac{4}{45} \alpha^2 + \frac{1}{210} \alpha^4 + \ldots \tag{15}$$

It is evident that as long as the leading term in α on the right side of Eqn 15 is much smaller than $^1/_3$ (which is the average zero-field value of $\cos^2 \theta$), the magnetic field will have no noticeable effect. When α becomes significant, there is a rapid super-linear increase with increasing $\boldsymbol{H}$.

At high fields, the value of $\langle \cos^2 \theta \rangle$ should approach unity, which corresponds to maximum alignment. The first term on the right side of Eqn 14 is a form of Dawson's integral which is tabulated[21] for values of $\alpha \geqslant 2$. The entire expression for $\langle \cos^2 \theta \rangle$ in Eqn 14 can be evaluated for the entire range of α from $0 \rightarrow \infty$ by computer. A plot of $\langle \cos^2 \theta \rangle$ as a function of α calculated *via* Eqn 14 is shown in Fig. 5. The calculated field dependence is remarkably similar to the curves shown in Fig. 1, and also to the curves of Chalazonitis *et al.*[22] who measured the degree of orientation of retinal rods in magnetic fields.

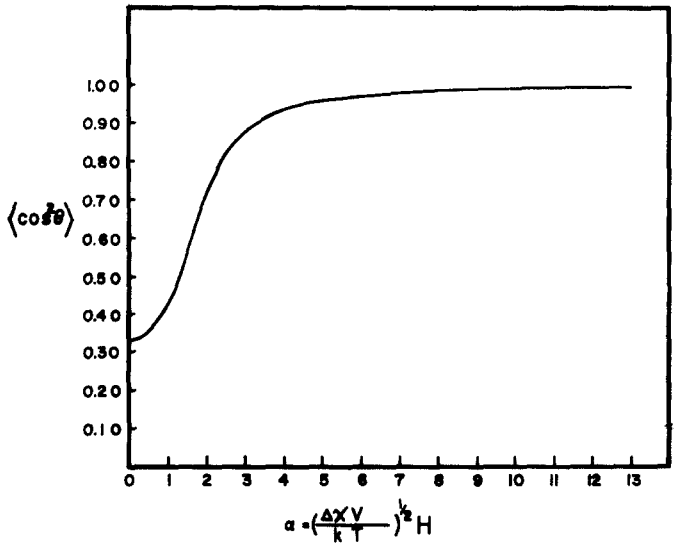

Fig. 5. Theoretical calculation of the magnetic field dependence of the fluorescence (see text).

Near-saturation occurs at values of $\alpha = 4$–5, or when the magnetic energy is about 16–25 times greater than kT. With *Chlorella pyrenoidosa* (395), this occurs at fields of approx. 10–11 kG. Taking the value of $\alpha \approx 3$ at the inflection point in Fig. 5, and finding that $H \approx 8$ kG at the experimental inflection point of *Chlorella pyrenoidosa* (395) in Fig. 1, $\Delta\chi V$ can be estimated crudely. It is about 10^{-20} cm^3. Since χ is of the order of 10^{-6} for organic molecules, an estimate of the "effective" cell volume V can be made if additional assumptions concerning $\Delta\chi$ are made. If the anisotropy is $\approx 10\%$, V is about 10^{-13} cm^3, which is smaller than the cell volume ($\approx 10^{-11}$ cm^3) by about a factor of 100. The smaller the anisotropy, the greater would have to be the effective volume V. This order of magnitude calculation shows that the range of numbers obtained for $\Delta\chi V$ are physically reasonable.

Theoretically, the maximum possible change due to a reorientation of the cells is by a factor of three. There are several reasons why the observed change should be much smaller. (1) The emitting dipole has some random orientation with respect to the magnetic axis. In this case an additional term would appear in Eqn 11, but the maximum possible change would be less than by a factor of three, even when the magnetic axis is completely aligned. (2) The chloroplast has a non-planar shape and (3) the pigment molecules within the lamellae are not perfectly oriented. These factors may account for the variability in the magnetic effect exhibited by different organisms and by different strains of the same species. The results with the broken and intact spinach chloroplasts indicate that the structure of the lamellae itself is important in determining the magnitude of the fluorescence change at maximum orientation. No effect was observed with *Phormidium*, which may be related to the fact that blue-green algae do not have chloroplasts and the pigments are concentrated in free grana. These comments, however, are of a speculative nature and the exact factors which play a role in the magnitude of the magneto-orientation effects await further elucidation.

On the orientation of chlorophyll

The mutual orientation of the bulk pigments, which give rise to the main absorption band at 675–680 nm, is considered to be of a low order. When single chloroplasts are viewed on edge, however, a longer wavelength-absorbing form appears to be oriented. When the chloroplasts are viewed with the light beam perpendicular to the plane of the lamellae, no orientation can be discerned within the plane. It was concluded that the plane of the porphyrin heads lie in or near the plane of the lamellae, but are unoriented within these planes[1–5,7].

In view of these conclusions, there appear to be at least two interpretations of the magnetic field induced dichroism and fluorescence polarization.

(1) The lamellae tend to orient with their planes perpendicular to H. The orientation of the chlorophyll molecules is not affected by the field. Since the planes of the porphyrin heads lie in or near the plane of the lamellae, this orientation would give rise to a more intense fluorescence and absorption polarized perpendicular to the field, as is observed. Such an orientation of the lamellae indicates that the plane of the porphyrin heads should also be oriented perpendicular to H. This is indeed the case as can be demonstrated by studies of magnetic dichroism in the shorter wavelength region[23] ($\lambda < 500$ nm) of the chlorophyll absorption *in vivo*.

(2) The lamellae, as well as the chlorophyll molecules, tend to orient in the

magnetic field. The field induced alignment of the pigments would not be sufficient to reduce depolarization by energy transfer as indicated experimentally. The fluorescence polarization disappears after removal of the magnetic field in a time which is characteristic of the cell rotation. Once the cells have randomized, the orientation of the chlorophyll molecules is unobservable. This may be the case even if they still possess some degree of orientation within the lamellae, because of a possible slower relaxation time of the pigments than of the whole cell.

Hypothesis 1, cell orientation only and no pigment reorientation, is adequate to explain all of the experimental facts reported here. Since we observe maximum dichroism within the main absorption band (the red shifts are caused by selective light scattering, which is strongest on the long wavelength side of the main "red" absorption peak[18]), Hypothesis 1 entails the conclusion that the main bulk of the pigments has a considerable degree of orientation. This is not in agreement with observations made on single chloroplasts under the microscope[1]. We have shown, however, that with suspensions of *Chlorella* cells, the magnetic dichroic maxima can be shifted to the red by viewing the light transmitted by cell suspensions at a small angle—an experimental condition which maximizes errors in the absorption measurements which are due to scattering effects. If the lamellae indeed line up perpendicular to $\boldsymbol{H}$ as we have suggested, light incident on the edge of the lamellae is preferentially scattered in a direction at 90° to the plane of the lamellae. This conclusion is reached from the observation that with *Chlorella* there is an increase in the amount of light scattered in a direction parallel to $\boldsymbol{H}$ when the field is turned on. When viewing single chloroplasts under the microscope, wavelength and electric vector orientation dependence of the light scattering should thus be considered. It is therefore possible that microscope examination of single chloroplasts viewed on edge are complicated by anisotropic light scattering effects which shift the apparent dichroic maxima to the red, just as we have observed with *Chlorella* suspensions[17].

Even though it is not necessary to invoke the reorientation of pigment molecules (Hypothesis 2) to explain our results, we have so far no evidence that this effect does, or does not occur. Additional experiments are needed to settle this issue, and are presently being planned.

The magnetic field induced orientation of whole photosynthetic organisms and spinach chloroplasts offer a means to study the fluorescence, absorption and light scattering of a large ensemble of suspended oriented cells. With *Chlorella pyrenoidosa*, a field of 10–11 kG, available with most ESR magnets, is sufficient to induce a degree of orientation close to saturation.

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