Proc. Nat. Acad. Sci. USA
Vol. 68, No. 6, pp. 1283-1285, June 1971

Magnetic Anisotropy and the Orientation of Retinal Rods in a Homogeneous Magnetic Field

(theoretical/retinal rod orientation)

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Communicated by S. Granick, April 14, 1971

ABSTRACT The reported orientation of retinal rods in a homogeneous magnetic field can be explained by the magnetic anisotropy of oriented molecules in the disc membranes of the rods.

The energy of a single rod as a function of orientation in the magnetic field, the time required for alignment of the rod in a viscous medium, and the fluctuations of orientation are calculated. Arguments that rhodopsin is the constituent responsible for the effect are given. The possibility of orientation due to inhomogeneity of the magnetic field is ruled out. The application of magnetic anisotropy as an experimental tool in biology is indicated.

Chalazonitis *et al.* [1] have reported the rotation and alignment of a suspension of rod outer segments from frog retinas in a constant and homogeneous magnetic field. In a magnetic field of 10 kG, the rods tend to orient themselves with their long axes parallel to the field. Membrane currents were invoked to explain the observation. However, this interpretation is not without difficulty. For instance, the equivalence of the two ends of a rod in the magnetic field can hardly be explained by the mechanism proposed, while it is to be expected by the alternative interpretation we present here.

We shall attempt to explain the phenomenon in terms of magnetic anisotropy of the oriented constituent molecules of the rod disc membranes. The argument involved is essentially that employed by Ornstein [2] in his "Swarm Theory" of the effect of magnetic field on the nematic mesophases of liquid crystals. Arnold *et al.* [3] also used the same argument to explain the orientation of long muscle fibers in a homogeneous magnetic field. Owing to the extraordinarily regular array of disc membranes [4] in the rod, any oriented anisotropy present will be summed up and thus reveal its presence through orientation in a magnetic field. Because of the lack of accurate data concerning magnetic anisotropies, only an order-of-magnitude estimation of the effect will be made. The possibility of orientation due to inhomogeneity of the magnetic field will also be considered.

In view of the regular stacking of the disc membranes in a rod, it is reasonable to assume an overall cylindrical symmetry of the molecular distribution and orientation. That the ordered membrane structure is essential is suggested by the observation [1] that osmotic shock or heating of the rods abolishes the orientation effect in a magnetic field. Thus, if magnetic anisotropy is present in the oriented molecular constituents of the disc membranes, macroscopically one of its principal axes of magnetic susceptibility will lie in the axial direction of the rod. From the assumed symmetry, the other two principal susceptibilities lying in the plane of the disc will be equal.

The total magnetic energy of a rod in a constant and homogeneous magnetic field of magnitude H can be easily shown to be

$$E = -(^1/_2)VH^2\{(\chi_a - \chi_r)\cos^2\theta + \chi_r\}, \qquad (1)$$

where χ_a and χ_r are the *axial* and the *radial* principal volume susceptibilities, respectively, of the oriented anisotropic molecules in a rod, and V is their effective volume, and θ is the angle between the direction of the magnetic field and the axis of the rod. Since the contributions from the isotropic and the randomly oriented anisotropic constituents of the rod are independent of the angle θ, they are neglected. We have also ignored the negligible contributions from the edges of the disc membranes.

Given the above energy expression, several different cases can occur. If $\chi_a > \chi_r > 0$ (paramagnetic) or $\chi_r < \chi_a < 0$ (diamagnetic), the rods have their stable equilibrium orientation at $\theta = 0°$ and $\theta = 180°$. If $\chi_r > \chi_a > 0$ (paramagnetic) or $\chi_a < \chi_r < 0$ (diamagnetic), the stable equilibrium orientation will be $\theta = 90°$. Since oriented paramagnetic molecules in the rod are unlikely to be sufficiently concentrated to overshadow the oriented diamagnetic molecules, and the observed orientation is $\theta = 0°$ or $180°$, we shall henceforth restrict our discussion to the case of diamagnetic anisotropy with $\chi_r < \chi_a < 0$. We will mention later a method of distinguishing between these possibilities.

A set of data of the rod outer segments of frogs is chosen for our calculation from the report of Nilsson [4] (Fig. 1). We tentatively take the effective volume V to be that occupied by the membranes, i.e., 9.0×10^{-10} cm^3, while the actual volume of a rod is 1.7×10^{-9} cm^3. If 1.2 is taken as the specific gravity of the rod (an upper limit obtained from their flotation property in a sucrose gradient [5]), the mass of a rod is found to be 2.0×10^{-9} g. We assume the values of susceptibilities to be of the order of a typical organic molecule, namely, 10^{-6}, and assume a difference of χ_a and χ_r of 1% (the data reported by Lonsdale [6] for long-chain alkyl molecules show anisotropies as large as 10%), so that $\chi_a - \chi_r = 10^{-8}$. The energy difference between a rod parallel to and one perpendicular to the magnetic field of 10 kG is 4.5×10^{-10} ergs; while the thermal energy at 25°C is of the order of $kT = 4.1 \times 10^{-14}$ ergs.

It is thus thermodynamically possible for such an orientation effect to occur. However, one must turn to the question

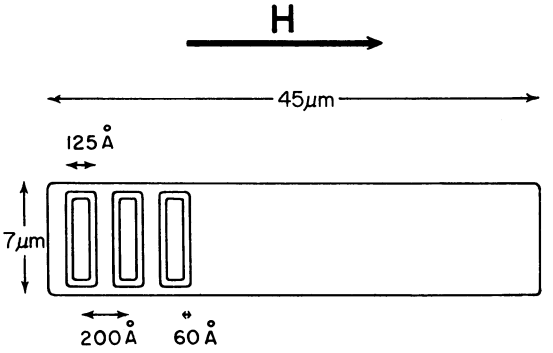

FIG. 1. A schematic diagram of the longitudinal section of a rod outer segment. The arrow indicates the direction of the magnetic field when the rod is in its equilibrium orientation. The physical dimensions are adapted from Nilsson [4]. Three discs with their bilayer membrane structure are shown (drawing not to scale; the thickness of the disc and the size of the space inside the discs are exaggerated). The total number of discs per rod is 1900. The total disc area is 1.5×10^5 μm².

of whether the effect is observable in a reasonably short time. This time should be less than 2 min, as this was claimed by Chalazonitis *et al.* [1] to be sufficiently long for observing the phenomenon.

We shall estimate the time, τ, required for a rod, initially at rest, to swing from $\theta = 89°$ to $\theta = 1°$, using the above-chosen parameters. It takes infinite time to swing from $\theta = 90°$ to $\theta = 0°$.

In setting up the equation of motion of a rod, we must also include the viscous damping effect of the Ringer solution, which involves a frictional torque proportional to the instantaneous angular velocity of the rod, the constant of proportionality being the rotatory frictional coefficient, ζ. We shall use a formula for a *slender* prolate ellipsoid simplified from one given by F. Perrin [7]:

$$\zeta = \frac{16\pi\eta a^3}{-3 + 6\ln(2a/b)} = 4.6 \times 10^{-10}\ \text{g-cm}^2\text{-sec}^{-1}, \quad (2)$$

where we put $a = (45/2)\mu\text{m}$, $b = (7/2)\mu\text{m}$, and the viscosity coefficient $\eta = 0.01$ poise, that of water at 20°C. The restoring torque is given by

$$-\partial E/\partial\theta = -(^1/_2)VH^2(\chi_a - \chi_r)\sin 2\theta, \quad (3)$$

and the relevant moment of inertia is $I = 3.4 \times 10^{-15}$ g-cm². The equation of motion is given by a nonlinear differential equation:

$$Id^2\theta/dt^2 + \zeta d\theta/dt + (^1/_2)VH^2(\chi_a - \chi_r)\sin 2\theta = 0. \quad (4)$$

By linear approximations to the restoring term, we obtain $\tau = (4.1 \pm 0.2)$ sec. Note also that since the first term in Eq. (4) is small compared with the others, we can obtain another good approximation for τ by dropping it:

$$\tau' = \frac{2\zeta}{VH^2(\chi_a - \chi_r)}\int_{1°}^{89°} \csc 2\theta d\theta = \frac{8.0\zeta}{VH^2(\chi_a - \chi_r)} = 4\ \text{sec}. \quad (5)$$

We also calculate the root-mean-square fluctuation $\Delta\theta_{\text{rms}}$ due to Brownian movement [8] in the absence of magnetic field:

$$\Delta\theta_{\text{rms}} = \sqrt{2kTt/\zeta} = 0.06\ \text{rad} = 3.4°, \quad (6)$$

for an interval of observation, $t = 5\tau' = 20$ sec, at 25°C. However, in the presence of a *high* magnetic field of 10 kG, such that $VH^2(\chi_a - \chi_r) \gg kT$, the small fluctuation observed in a plane containing the magnetic field vector is given by

$$\Delta\theta_{\text{rms}} = \sqrt{kT/\{VH^2(\chi_a - \chi_r)\}} = 0.007\ \text{rad} = 0.4°, \quad (7)$$

using the given parameters.

Thus, the orientation effect can be fairly accurately detected in a reasonably short time of observation.

From Eq. (5), the orientation time is seen to be inversely proportional to the square of the field strength. As a consequence, at sufficiently low field strengths the effect is unobservable because of prolonged orientation time and increased thermal fluctuation. This might account for the incomplete orientation observed by Chalazonitis *et al.* [1] at low field strengths. Because of the summing effect of the oriented molecules in the rod, extremely small (e.g., 0.01%) magnetic anisotropy can be detected. For a given magnetic anisotropy and applied magnetic field, the ability to detect an orientation effect depends on the number of oriented anisotropic molecules in the rod.

So far we have made no reference to any particular molecular constituent of the rod in our calculation. The most likely candidates for the oriented anisotropic molecules are phospholipid molecules and rhodopsin molecules in the disc membranes. The phospholipid molecules in the disc membranes possess a macroscopically cylindrical symmetry because of their orientation in the bilayer structures. Since the dichroism in intact rod outer segments has been associated with rhodopsin [9], the rhodopsin molecules must be oriented in the disc membranes. *In situ* studies of the dichroism with light perpendicular to the rod axis by Liebman [10] show that the transition moment of rhodopsin must be largely confined to the plane of the discs. Studies of dichroism with light parallel to the rod axis by Brown [11] and by Cone [12] show that rhodopsin molecules are free to rotate in the disc about an axis perpendicular to the discs. Hence, the orientation of rhodopsin molecules in the disc membranes possesses an overall cylindrical symmetry. By inserting the known diamagnetic susceptibilities of stearic acid [6] as an approximation to the phospholipid, one predicts an orientation perpendicular to the field, i.e., contrary to that observed. This result, and the observation of a light-induced orientation effect [1], suggest that rhodopsin may be the oriented constituent in the rod that contributes most to the magnetic anisotropy. If this is so, we predict that its diamagnetic susceptibility is stronger in the plane of the discs than in the axial direction, i.e., $\chi_r < \chi_a < 0$. In this case, the effective volume of rhodopsin in a rod is estimated from the reported rhodopsin concentration [10] (2.5 mM) and the known size of a rhodopsin molecule (40–50 Å in diameter) [5] to be 1.0×10^{-10} cm³. An alternative estimate, by use of the data of the size of the unit cells of the square array of rhodopsin particles [5], gives the same approximate value. Although this value is 10% of that which we have used for our calculation, the orientation effect could still be readily observed.

One may raise the question whether an inhomogeneity of the magnetic field might have an orientation effect on a magnetically isotropic rod, because of its shape. We shall consider the case of a magnetic field with a linear gradient in one direction, which does not necessarily coincide with the direction of the field. We find that the total magnetic energy of a rod in Ringer solution is given by

$$E = -(1/2)V(\chi - \chi_w)\{H_0^2 + (1/12)(Kl \cos \theta)^2\}, \qquad (8)$$

where χ and χ_w are the volume susceptibilities of rods and water, respectively, H_0 is the magnetic field strength at the center of the rod, K is the linear gradient, θ is the angle between the rod axis and the direction of the gradient, and l and V are the length and the volume of the rod, respectively.

This result shows that a rod will translate along the increasing field gradient and also rotate so that it becomes parallel to the gradient if it is less diamagnetic than water or even paramagnetic; it will translate along the decreasing field gradient and also rotate so that it becomes perpendicular to the gradient if it is more diamagnetic than water.

For $H_0 = 10^4$ G and $K = 1$ G/cm, a degree of homogeneity easily surpassed in the laboratory, if we put $\chi = -0.66 \times 10^{-6}$, that of a typical organic molecule, and $\chi_w = -0.72 \times 10^{-6}$, we find that the energy difference between parallel and perpendicular orientation is 0.9×10^{-22} ergs, which is negligibly small compared with the thermal energy, of the order of 4.1×10^{-14} ergs at 25°C. Thus, with some care to avoid sources of inhomogeneity, this should not be a problem. On the other hand, by observing the orientation and the translation of the rods in a sufficiently inhomogeneous magnetic field, one can tell whether the rod is overall diamagnetic or paramagnetic.

The confirmation of the possible presence of anisotropy of the rod outer segments lies in a straightforward measurement of the susceptibilities in different directions on a regularly packed sample of rods. Such packing may be obtained by means of centrifugation, as was performed in the study of Blasie *et al.* [5]. On the other hand, observation of the time course of the orientation effect provides a way to measure the overall quantity $V(\chi_a - \chi_r)$ for the rods. The observation may also provide a method of studying the light-induced conformation change of rhodopsin molecules.

Finally, during the preparation of our manuscript, Dr. Geacintov brought to our attention the observation of the effect of a magnetic field on the measurement of chlorophyll *a* fluorescence yield of intact *Chlorella* cells [13]. Our calculation suggests that at least part of the effect observed can be attributed to induced orientation of the chloroplasts because of the regular stacking of the grana. It is possible that magnetic anisotropies may be very useful not only in preparing oriented biological samples, but also in determining the values of their anisotropic susceptibilities.

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