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MOTION OF MAGNETOTACTIC MICROORGANISMS

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SUMMARY

Magnetic moments for different magnetotactic microorganisms are obtained by electron microscopic analyses and studies of cell motion by optical microscopy. The results are analysed in terms of a model due to C. Bean. The considerations presented suggest that magnetotaxis is an efficient mechanism for orientation only if the time for reorientation is smaller than the mean time of environmental perturbations.

INTRODUCTION

It has been shown that the geomagnetic field could play a role in the orientation, navigation and homing of a great number of organisms (Palmer, 1963; Schmidt-Koenig & Keeton, 1978). Until recently the mechanism of perception of magnetic fields was unknown, although the behavioural effects of weak magnetic fields on different organisms have been studied (Barnothy, 1969). The strength of the geomagnetic field is of the order of 50 μT, varying from 25 μT (in the South Atlantic geomagnetic anomaly) to 60 μT at the poles, and the inclination varies from near 80° at the North magnetic pole to −80° at the South magnetic pole, with null inclination at the geomagnetic equator. The interesting regions are those where the magnitude of the field is very small and/or near the geomagnetic equator and where it is possible to observe how sensible is the response of microorganisms to the local geomagnetic field.

There are at least two different mechanisms by which organisms can detect the geomagnetic field (Frankel, 1984). Certain organisms are capable of detecting weak voltage gradients. They are thus able to detect the field by magnetic induction, as shown by Kalmijn (1978) for elasmobranchs. The other mechanism is based on intracellular, permanently magnetic material as in magnetotactic organisms (Blakemore, 1975, 1982; Frankel, 1984; Kirschvink, 1982). In the particular case of magnetotactic microorganisms, the magnetic material consists of biomineralized single-magnetic-domain magnetite (Fe_3O_4) particles (Frankel, Blakemore & Wolfe, 1979; Towe & Moench, 1981; Mann, Frankel & Blakemore, 1984). The magnetic material is found inside the magnetosome, a specific organelle responsible for the

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biomineralization of magnetite. The particles are often aligned in a chain which imparts a permanent magnetic dipole moment to the cell. The dipole moment interacts with the external magnetic field, producing a torque which tends to align the moment along the field lines. This response is passive in the sense that killed microorganisms are also oriented in the field.

We report here results of electron and optical microscopy studies of magnetotactic microorganisms from brackish marine and freshwater environments in different regions of the city of Rio de Janeiro. We have previously described a variety of morphological types of magnetotactic microorganisms from these environments (Esquivel *et al.* 1983; Farina, Lins de Barros, Esquivel & Danon, 1983).

We have also studied the movement of the microorganisms in a laboratory magnetic field, measuring the swimming velocities (v), and times and radii of 'U-turns' following field reversals. The results are analysed in terms of a model proposed by Charles P. Bean (Frankel, 1984; see Appendix).

The magnetic moment values for different microorganisms are estimated from U-turn analyses (m_u) and/or from electron micrographs of the magnetite chain (m_{em}).

METHODS

Techniques and preparation of samples

Samples with sediments and water were collected in three places on different dates, between 20 and 60 cm deep. Rodrigo de Freitas coastal lagoon is located in Rio de Janeiro city, in the urbanized metropolitan area, where waste disposal has overloaded the normal ecosystem. The organically polluted waters are in the saprobic biological zone, where there is a bacterial population, decomposing the organic matter, and the planktonic blue green algae *Anabaena* and *Oscillatoria*. Hydrogen sulphide is common in the bottom of this lagoon and combines with the iron salts and oxides to form sulphides, which colour the anaerobic layer black. Marine waters from Guanabara bay also contain a very high level of sewage and industrial pollutants. Fresh waters, in contrast, are from small rivers located in an ecological reserve in the Rio de Janeiro metropolitan area. Despite the high pollution in Rodrigo de Freitas lagoon and Guanabara bay, we have observed no significant alteration in viscosity. After 4 or 5 days in the laboratory at ambient temperature, the concentrations of magnetotactic microorganisms had increased to up to 10 000 cells cm^{-3}.

The magnetotactic microorganisms were further concentrated by a bar magnet or Helmholtz coil. Samples were placed in glass tubes 10 cm long and 3 cm in diameter ending as micropipettes. The magnets were positioned so the magnetotactic microorganisms would swim to and concentrate in the micropipette. A few minutes after attachment of the magnet, living magnetotactic microorganisms could be collected without sediment or other microorganisms.

Preparations for TEM (transmission electron microscopy) were made by placing living magnetotactic microorganisms on copper grids (400 mesh) covered with collodion film: it is possible to observe the magnetic response on these grids by using

optical microscopy. These cells were fixed in osmium tetroxide vapour without carbon reinforcement and the excess liquid was drawn off the grid surface. The preparations were allowed to dry in air and were observed in a JEOL 100CX electron microscope with maximum voltage filament (100 kV).

Optical studies were made using a Leitz-Ortholux microscope with objectives of 10–100× and eyepieces of 10–40× coupled to photographic, cinematographic or video systems. Natural water samples with sediments were placed directly on the slide. The constant magnetic field was obtained by a pair of Helmholtz coils calibrated by a digital magnetometer (DM 2220, Schonstedt Instrument Company). We used clear ground lighting or dark field illumination to observe or record the movement of magnetotactic microorganisms as a function of the applied field. To reduce contributions due to the geomagnetic field we oriented the microscope so that the geomagnetic field was parallel to the field of the Helmholtz coils.

The cellular magnetic moment can be estimated from electron microscope images of the linear chain if we assume that these regions are shaped as hexagonal prisms and are composed of 80 % magnetite (Fe_3O_4) (Towe & Moench, 1981; Blakemore, 1982; Matsuda *et al.* 1983). Magnetic moment = (volume of all particles in the linear chain) × (saturation magnetization per unit volume, $4{\cdot}8\times10^5$ J m^{-3} T^{-1} for magnetite). However, this procedure can only be used when high resolution images of the interior of the cell are obtained and when the chain is linear.

Another method of measuring the total magnetic moment of a microorganism is based on analysis of the U-turn as suggested by Bean (see Appendix). It relies upon the response of the organisms to the reversal of the magnetic field. We have observed that in a constant magnetic field the magnetotactic microorganisms swim in an approximately helical trajectory along the field lines. The stronger the field, the tighter is the helical turn. This trajectory is approximately a straight line and when the field is suddenly reversed, the microorganisms are subjected to a torque which reverses the direction of movement, resulting in an approximate U-trajectory (Fig. 1).

According to the Bean model (see Appendix) the reversal time (τ) and the diameter (L) of the U-turn depend upon the total magnetic moment (m) of the organism and are given by:

$$\tau = \frac{8\pi\eta R^3}{mB_o} \ln\left(\frac{2mB_o}{kT}\right) \tag{1}$$

$$\text{and} \quad L = \frac{8\pi^2 R^3 v\eta}{mB_o}, \tag{2}$$

where B_o is the magnetic field, R is the radius of the cell, η is the viscosity ($\eta_{H_2O} = 10^{-2}$ poise; 1 poise = 10^{-1} Pa.s), k is the Boltzmann constant and T is the temperature.

Equations 1 and 2 are obtained assuming no influence of flagellar movement. Thus, the U-turn method is a general one for estimating the magnetic moment.

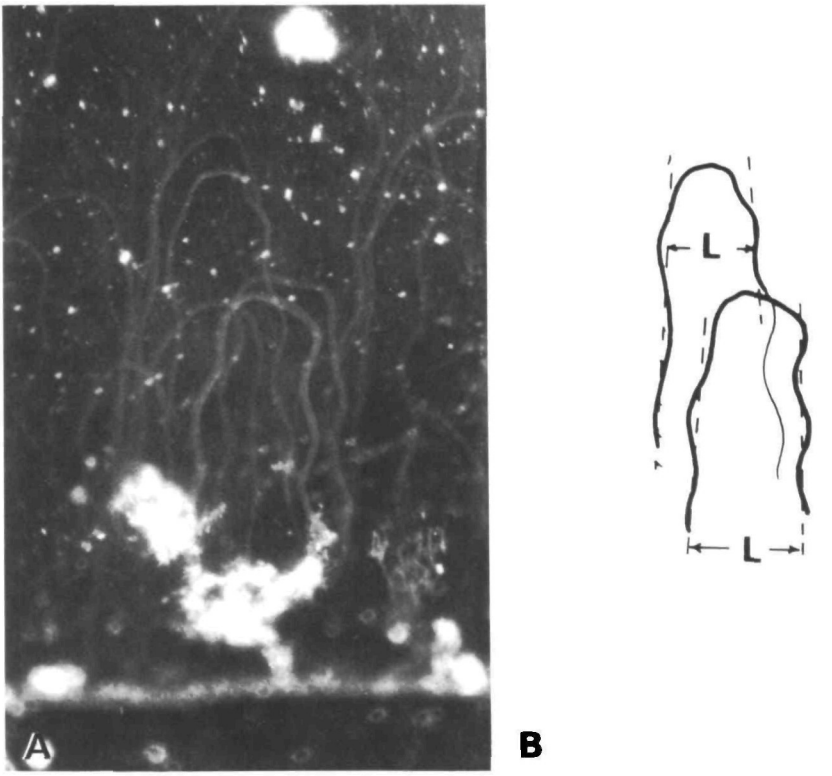

Fig. 1. (A) Dark field image from optical microscopy of the trajectories of various magnetotactic microorganisms (number 5 in Table 1). The photograph was obtained with an exposure of about 1 s. (B) Graphic representation of the U-turn obtained from the photograph. L, diameter of U-turn.

Information about magnetic moment was also obtained by using pure cultures (Rosenblatt, Torres de Araujo & Frankel, 1982*a*,*b*).

RESULTS

Table 1 presents the principal results and some theoretical estimates of the relevant parameters from the analysis of the movement and magnetic properties of the magnetotactic microorganisms. These numerical values are obtained by averaging the results for many samples (more than 20) and for different individual microorganisms of the same type. With this procedure we hope that the estimated values will be representative of each population.

In measurements of the U-turn we used $B_o = 930\,\mu T$, because this field is sufficiently high for the Bean model to be a good approximation for the movement (see Appendix).

We have measured L, v and R to obtain an estimate of m_u. Then we calculated, using equation 1, the reversal time, τ_u, and compared it with the measured value,

Table 1. *Some characteristics of the magnetotactic microorganisms found in Rio de Janeiro, including the site where the sediments were recovered and a picture obtained with optical microscopy*

Site	Scale bar = 5 μm	R (μm)	N	v (μm s^{-1})	τ_u (s)	τ_{exp} (s)	L (μm)	$m_{E.M.}$ 10^{-15} Am^2	m_u 10^{-15} Am^2	mB_o/kT	τ_o (s)
Fresh water	1	0·5	5	100	0·08	0·05	3	0·5	0·3	3	0·5
	2	1	7	50	0·3	0·3	8	0·7	0·5	4	3·6
	3	1·5×2·5 (~2·0)	—	12	0·9	1·3	8	—	1	6	20
Rio de Freitas	4	0·9	10	—	—	0·09	—	1·4	—	8	1·5
	5	2·5	>1000	40	1·3	1·4	30	—	2·4	17	25
Guanabara bay	6	0·9	10	—	—	0·1	—	1·3	—	8	1·5
	7	2·5	—	70	0·3	0·4	11	—	8	48	8·9
	8	5×9 (~7·5)	—	30	0·4	2·1	20	—	54	326	50

R, mean radius of the organism obtained from optical and/or electron microscopy.
N, mean number of magnetite particles found inside the microorganism by TEM.
v, mean velocity at B = 930 μT.
L, mean diameter of the U-turn at B = 930 μT.
τ_{exp}, mean reversal time of the U-turn measured at B = 930 μT.
τ_u, reversal time calculated from equation 1 with B = 930 μT.
m, estimated magnetic moment: EM from electron microscopy, u, from Bean model.
mB_o/kT, ratio between the magnetic and thermal energy in local geomagnetic field (B_o = 25 μT).
τ_o, reversal time for the U-turn calculated from equation 1 with B_o = 25 μT.

τ_{exp}, in the same field. τ_u and τ_{exp} are in good agreement, showing that the m_u is a good estimate for the total magnetic moment.

Table 1 also shows that N, the number of particles, and m are increasing functions of R (or, what is more significant, the mean volume αR^3). One important characteristic is that the ratio mB_o/kT depends upon the volume of the microorganism. For the smallest observed magnetotactic microorganisms this ratio is about 5, i.e. the average orientation is far from the saturation condition (see Appendix). However, the largest magnetotactic microorganisms have a ratio of mB_o/kT that is much greater than necessary to guarantee a total orientation in the geomagnetic field.

DISCUSSION

The results presented in this paper permit us to look at magnetotaxis in a new way.

Magnetotactic microorganisms at low concentrations (less than 10^5 cells cm^{-3}) can be treated as non-interacting magnetic dipoles. The average alignment of a magnetic dipole subjected to a field B_o is given by:

$$<\cos\theta> = L\left(\frac{mB_o}{kT}\right), \tag{3}$$

where $L(x) = \coth x + 1/x$ is the Langevin function of classic paramagnetism and mB_o/kT is the ratio of the magnetic interaction energy to the thermal energy. For $x \ll 1$, $L \to 0$, and so the dipoles are weakly aligned in the field, while for $x > 10$, $L \sim 1$ and we have an almost complete alignment ($<\cos\theta> \sim 1$).

Usually, it is considered that the conditions for magnetotactic orientation are obtained when $mB_o/kT > 10$ (or, the average orientation is given by $<\cos\theta> \sim 1$) (Frankel & Blakemore, 1980). Since the migration velocity is given by $v = v_o <\cos\theta>$, $mB_o/kT > 10$ means that $v \sim v_o$. However, when the ratio mB_o/kT is of the order of 1, the average migration velocity is about 30% of the instantaneous velocity. It has been suggested that downward directed motion enables magnetotactic bacteria to avoid the toxic effects of high O_2 concentrations at the surface (Blakemore, 1982). Consequently, in the geomagnetic field, the vertical component of the migration velocity, which gives the velocity with which the microorganisms swim to the bottom is:

$$v_V = v_o <\cos\theta> \sin I \sim 0{\cdot}3 v_o \sin I, \tag{4}$$

where I is the inclination of the geomagnetic field. Thus, in Rio de Janeiro ($B_o = 25\,\mu T$, $I \sim 25°$) for a microorganism with $mB_o/kT = 1$ ($m_m = 1{\cdot}6 \times 10^{-16}$ Am^2) we obtain:

$$v_V \sim 0{\cdot}1 v_o.$$

This result indicates that even in this case there is a biological advantage, i.e. magnetotaxis can be a more efficient mechanism than chemotaxis in producing a

displacement towards the bottom (Frankel, 1982). However, North-seeking as well as South-seeking bacteria are observed in roughly equal numbers in the same sediment samples collected near the geomagnetic equator (Frankel *et al.* 1981). This finding implies that magnetotaxis can be important even without downward directed motion and that bacteria would be able to migrate rapidly over long distances. In Table 1, the estimates of m result in $mB_o/kT > 3$ for the observed microorganisms.

On the other hand, the microorganism that uses the geomagnetic field as an orientation mechanism must have some means of responding efficiently to variations of this field, or to variations of its orientation in this field due to other environmental perturbations.

If we ignore flagellar motion, magnetotactic microorganisms behave like dipole particles with diameters in the range of 1 to a few microns. A particle with a diameter of about 1 μm is randomly disoriented by the medium (Brownian motion). Particles of more than a few microns are not so perturbed by statistical fluctuations of the medium (Purcell, 1977). In this range, other factors can be more important in disorienting these particles. Observations of natural samples analysed by us show very rich populations of different species of microorganisms which swim in different ways. These movements occur simultaneously and make possible random collision between microorganisms.

The movement of a specific microorganism can be perturbed by collisions with other organisms as well as by perturbations like current flow, statistical fluctuations, flagellar motion etc., and we can define a characteristic mean time between two successive perturbations for this microorganism. Hence, if there are other environmental perturbations that make the microorganisms deviate from their normal trajectory, the orientation in the field has to be performed in a time shorter than the mean time between two consecutive perturbations.

The above considerations show that to guarantee magnetic orientation it is necessary that the magnetic interaction energy be greater than the thermal disorder energy, i.e. $mB_o/kT > 1$. This condition is satisfied for magnetic moments of the order of $m_m = 1{\cdot}6 \times 10^{-16}\,Am^2$ in the local geomagnetic field ($B_o = 25\,\mu T$). The reversal time (τ) increases with the cube of the radius of the microorganism for a fixed value of m (equation 1). For larger organisms, the rapid increase of τ at constant m would make the response to magnetic stimuli inefficient. Curve A (Fig. 2) shows the values of τ calculated for m_m and $B_o = 25\,\mu T$. As shown in Fig. 2, we note that the reversal time (τ_o in Table 1), calculated with the estimated magnetic moment, also increases with R^3, but with a lower rate than the curve A. We think that the reversal time must have an upper limit to guarantee an efficient orientation.

These considerations lead us to suggest that there is an upper limit to the size of organisms for which magnetotaxis by passive orientation would be efficient.

Thus, magnetotaxis seems to be an orientation mechanism that is effective when (a) the magnetic interaction energy is much greater than the thermal energy and (b) the time interval necessary for the torque produced by the geomagnetic field to orientate the microorganism is much smaller than the mean time of perturbations that occur in their habitat.

APPENDIX

In this appendix the Bean model for the movement of a magnetotactic bacterium is described. Magnetotactic bacteria can be treated as an 'ensemble' of non-interacting magnetic dipoles. The flagellum provides the force necessary for forward motion. Since the flow is complete lamellar (i.e. the Reynolds number is very low), all inertial terms can be neglected. In a good approximation the flagellar force is equilibrated by the viscous force, and in 'high' magnetic fields (over 500 μT) a bacterium swims with a linear trajectory. In this sense we can treat a magnetotactic bacterium as a magnetic dipole moving with constant velocity, v_o, in the medium, subjected only to thermal

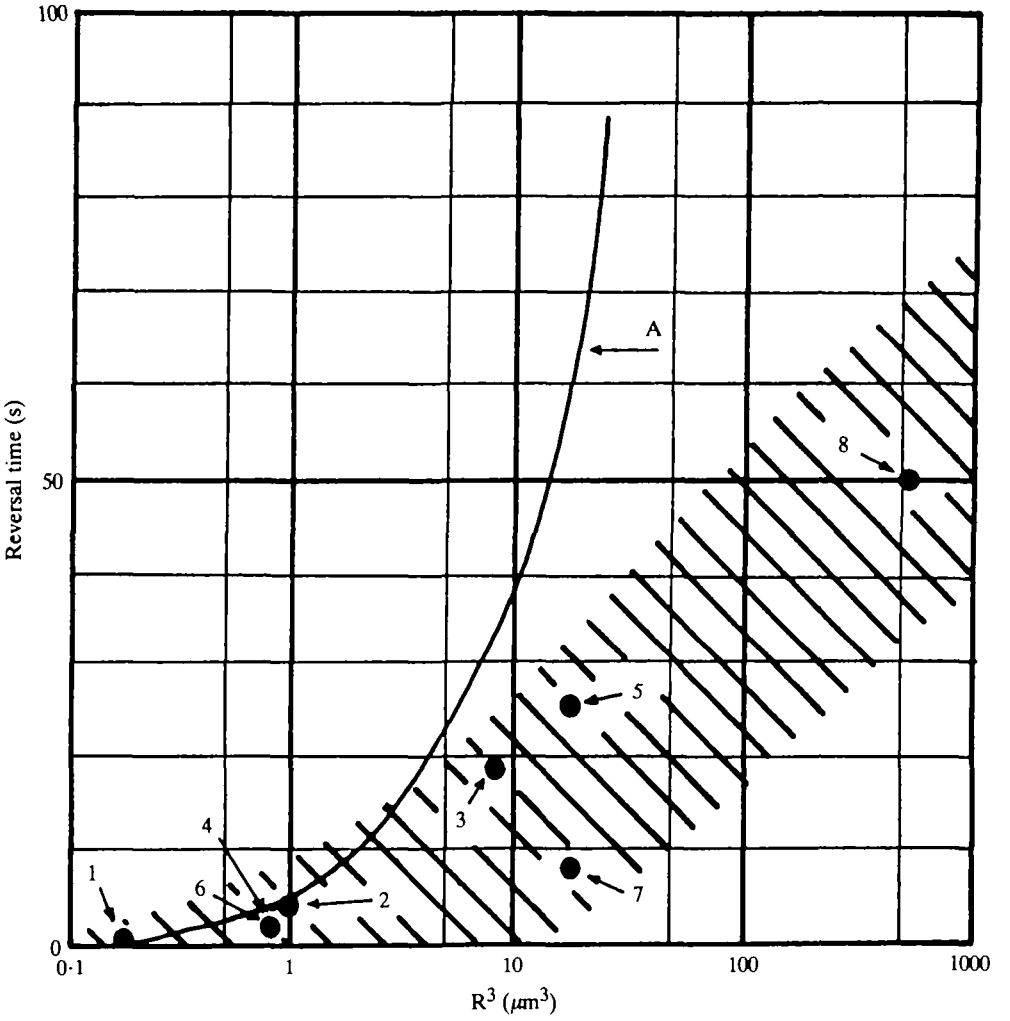

Fig. 2. Relationship between reversal time (τ) in the local geomagnetic field ($B_o = 25\ \mu$T) and volume of some magnetotactic microorganisms. Curve A, assuming $m = 1{\cdot}6 \times 10^{-16}$ Am^2 for all the microorganisms and τ calculated from equation 1. ●, mean reversal times obtained from the estimated values of m (Table 1). The cross-hatched region is the expected region for the reversal time.

perturbation. The average orientation is given by the classic theory of paramagnetism:

$$<\cos\theta> = L(mB_o/kT),$$

where L is the Langevin function defined in the text, and θ is the angle between the direction of the magnetic field and the magnetic dipole moment. The migration velocity, v, is then:

$$v = v_o <\cos\theta>.$$

The saturation condition occurs when $mB_o/kT > 10$, which means that $v > 0{\cdot}9v_o$.

The forces acting on the bacterium are basically the flagellar force, which is equilibrated by the viscous forces of the medium, random forces and perturbations due to currents etc. If we neglect the contribution of all these forces, except for the thermal perturbation, the equation of motion in the centre-of-mass coordinate system is:

$$\text{Torque from field} + \text{viscous drag torque} = 0.$$

Assuming that the bacterium is a perfect sphere of radius R with dipole moment m:

$$mB_o \sin\theta - 8\pi\eta R^3\left(\frac{d\theta}{dt}\right) = 0. \tag{A1}$$

Equation A1 can be integrated exactly and we obtain:

$$\ln(\tan\theta/2) = \frac{t}{T_o} + \ln\tan\left(\frac{\theta_i}{2}\right), \tag{A2}$$

where $T_o = 8\pi\eta R^3/mB_o$ and θ_i is the initial orientation angle.

When the field is suddenly reversed (or, which is completely equivalent, the bacterium rotates in relation to the field), $\theta_i = \pi$ and equation A2 diverges.

This difficulty can be removed if θ_i is taken to be small (i.e. $\tan(\theta_i/2) \sim \theta_i/2$) and equal to the average angle obtained from the Langevin function, i.e. $\theta_i \sim (2kT/mB_o)^{1/2}$ for $mB_o \gg kT$. Then:

$$\ln\tan\left(\frac{\theta_i}{2}\right) \sim \ln\left(\frac{\theta_i}{2}\right) \sim \ln\left(\frac{2kT}{mB_o}\right)^{1/2}.$$

The resulting expression for the reversal time is:

$$\tau = \frac{8\pi\eta R^3}{mB_o}\ln\left(\frac{2mB_o}{kT}\right). \tag{A3}$$

τ does not depend on the velocity v_o and is directly proportional to R^3.

In this situation the bacterium performs a U-trajectory and the diameter of this curve, L, can be obtained by:

$$L = \int_0^\infty v_T dt = \int_0^\infty v_o \sin\theta \, dt.$$

Equation A1 gives:

$$\sin\theta = \frac{8\pi\eta R^3}{mB_o} \frac{d\theta}{dt}.$$

Then:

$$L = \frac{8\pi^2\eta R^3 v_o}{mB_o}.$$

Thus, L is inversely proportional to m and B_o and is directly proportional to R^3 and v_o.

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REFERENCES

BARNOTHY, M. F. (ed.) (1969). *Biological Effects of Magnetic Fields,* vol. 2. New York: Plenum Press.

BLAKEMORE, R. P. (1975). Magnetotactic bacteria. *Science* **190**, 377–379.

BLAKEMORE, R. P. (1982). Magnetotactic bacteria. *A. Rev. Microbiol.* **36**, 217–238.

ESQUIVEL, D. M. S., LINS DE BARROS, H. G. P., FARINA, M., ARAGÃO, P. H. A. & DANON, J. (1983). Microorganisms magnetotactiques de la region de Rio de Janeiro. *Biol. Cell.* **47**, 227–234.

FARINA, M., LINS DE BARROS, H. G. P., ESQUIVEL, D. M. S. & DANON, J. (1983). Ultrastructure of a magnetotactic microorganism. *Biol. Cell.* **48**, 85–86.

FRANKEL, R. B. (1982). Magnetotactic bacteria. *Comm. mol. cell. Biophys.* **1**, 293–310.

FRANKEL, R. B. (1984). Magnetic guidance of microorganisms. *A. Rev. Biophys. Bioeng.* **13**, 85–103.

FRANKEL, R. B. & BLAKEMORE, R. P. (1980). Navigational compass in magnetic bacteria. *J. Magn. magn. Matters* **15–18**, 1562–1564.

FRANKEL, R. B., BLAKEMORE, R. P., TORRES DE ARAUJO, F. F., ESQUIVEL, D. M. S. & DANON, J. (1981). Magnetotactic bacteria at the geomagnetic equator. *Science* **212**, 1269–1270.

FRANKEL, R. B., BLAKEMORE, R. P. & WOLFE, R. S. (1979). Magnetite in freshwater magnetotactic bacteria. *Science* **203**, 1355–1356.

KALMIJN, AD. J. (1978). Experimental evidence of geomagnetic orientation in elasmobranch fishes. In *Animal Migration, Navigation, and Homing* (ed. K. Schmidt-Koenig & W. T. Keeton). Berlin, Heidelberg, New York: Springer-Verlag.

KIRSCHVINK, J. L. (1982). Birds, bees and magnetism. *Trends Neurosci.* **5**, 160–167.

MANN, S., FRANKEL, R. B. & BLAKEMORE, R. P. (1984). Structure, morphology and crystal growth of bacterial magnetite. *Nature, Lond.* **310**, 405–407.

MATSUDA, T., ENDO, J., OSAKABE, N., TONOMURA, A. & ARII, T. (1983). Morphology and structure of biogenic magnetite particles. *Nature, Lond.* **302**, 411–412.

PALMER, J. D. (1963). Organismic spatial orientation in very weak magnetic fields. *Nature, Lond.* **198**, 1061–1062.

PURCELL, E. M. (1977). Life at low Reynolds number. *Am. J. Physiol.* **45**, 3–11.

ROSENBLATT, C., TORRES DE ARAUJO, F. F. & FRANKEL, R. B. (1982*a*). Light scattering determination of magnetic moments of magnetotactic bacteria. *J. appl. Physiol.* **53**, 2727–2729.

ROSENBLATT, C., TORRES DE ARAUJO, F. F. & FRANKEL, R. B. (1982*b*). Birefringence determination of magnetic moments of magnetotactic bacteria. *Biophys. J.* **40**, 83–85.

SCHMIDT-KOENIG, K. & KEETON, W. T. (eds) (1978). *Animal Migration, Navigation, and Homing*. Berlin, Heidelberg, New York: Springer-Verlag.

TOWE, K. M. & MOENCH, T. T. (1981). Electron-optical characterization of bacterial magnetite. *Earth planet. Sci. Lett.* **52**, 213–220.