ISSN 0029-3865

CBPF-NF-011/85

MOTION OF MAGNETOTACTIC MICROORGANISMS

by

D.M.S. Esquivel and H.G.P. Lins de Barros

Centro Brasileiro de Pesquisas Físicas - CNPq/CBPF
Rua Dr. Xavier Sigaud, 150
22290 - Rio de Janeiro, RJ - Brasil

ERRATA

change "pg. 4" to "pg. 5"

change "pg. 5" to "pg. 4"

pg. 16 - change "E.M. from electron microscopy"

to

"E.M. from electron microscopy, where we assume that the crystals are composed by 80% of pure magnetite (Blakemore, 1982)".

ERRATA

change "pg. 4" to "pg. 5"

change "pg. 5" to "pg. 4"

pg. 16 - change "E.M. from electron microscopy"

to

"E.M. from electron microscopy, where we assume that the crystals are composed by 80% of pure magnetite (Blakemore, 1982)".

ABSTRACT

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

Key-words: Magnetotaxy; Microorganisms; Magnetism; Magnetic moment; Behaviour.

INTRODUCTION

The study of living beings has shown that many environmental stimuli have significant effects on behavior (Palmer, 1976; Gould, 1982). Among these the geomagnetic field could also play a role in the orientation, navigation and homing of great number of organisms (Palmer, 1963; Schmidt-Koenig and Keeton, 1978). Until recently the mechanism of perception of magnetic fields was unknown, although behavioral effects of weak magnetic fields on different organisms were studied (Barnothy, 1969). The strength of the geomagnetic field is of the order of 0.5G, varying from 0.25G (in the South Atlantic geomagnetic anomaly) to 0.6G at the poles, while the inclination varies from near 80º at the North magnetic pole to -80º at the South magnetic pole, with null inclination at the geomagnetic equator. The organisms that detect the geomagnetic field are apparently sensitive to these variations. The lower limit for the magnetic response happens at the regions where the magnitude of the field is very small, and also near the geomagnetic equator, where the vertical component of the field is very small.

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 the elasmobranchs. The other mechanism is based on intracellular magnetic material as in magnetotactic microorganisms, and other organisms as well (Frankel, 1984; Kirschvink, 1982). In the particular case of magnetotactic microorganisms, the magnetic material consists of biomineralized single-magnetic-do-

main magnetite (Fe_3O_4) particles (Blakemore, 1975; Frankel et al., 1979; Towe and Moench, 1981). 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 fresh water 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 et al., 1983).

We have also studied the movement of the microorganisms in laboratory magnetic fields measuring the swimming velocities V, and times and radii of "U-turns" following field reversals. The results are analyzed in terms of a model due to C.P. Bean (personal communications, Appendix A).

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

- Techniques and preparation of samples

Samples of sediment and water were collected at different dates in waters between 20 and 60 cm deep. The waters are very polluted with organic material and contain a great diversity and quantity of microorganisms. After four or five days in the laboratory at ambient temperature the samples had enriched to concentrations of magnetotactic microorganisms 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 micropipets. The magnets were positioned so the magnetotactic microorganisms would swim to and concentrate in the micropipet. A few minutes after attaching the magnet rich samples of living magnetotactic microorganisms could be collected without sediment or other microorganisms.

Preparations for TEM (transmission electron microscopy) were made by placing the organisms on a grid covered with collodium film and fixing with Osmium tetroxide vapour. These preparations were observed in a JEOL 100 CX electron microscope.

Optical studies were made using a Leitz-Ortholux microscope with objectives of 10 to 100X and oculars with 10 to 40X, coupled to cinematographic or video systems. It was possible to observe and record the movement of the magnetotactic microorganisms in the samples.

The cellular magnetic moment can be estimated from the electron microscope images at the linear chain of magnetite particles:

mag. mom. = (vol. of all particles in the linear chain) x (saturation magnetization per unit volume (480 erg/Gcm^3 for magnetite))

where we assume 80% pure magnetite (Blackmore, 1982).

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 microorganisms is based on analysis of the U-turn as suggested by Bean. 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 (Figure 1).

According to the Bean model 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:

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

$$L = \frac{8\pi^2 R^3 v\eta}{mB_o} , \qquad (2)$$

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

Equations (1) and (2) are obtained assuming no influence of flagellar movement or from any particular characteristics of the microorganism. Thus, the U-turn method is a general one for estimating the magnetic moment. Other means to obtain information about the magnetic moment are possible only when pure cultures are available (Rosenblatt et al., 1982a-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 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 = 9.3G because this field is high enough so that the Bean model is a good approximation for the movement. Measurements in smaller fields (less 5G), showed that the effects due to flagellar movement, convection currents, etc..., were as important as the magnetic interaction between the field and the microorganism and the trajectories were distorted.

We have measured L, v and R to obtain an estimate of m_u. Then we calculated, using eq.(1), the reversal time, τ_u , and compared it with the measured value, τ_{exp}, at 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 show that N, the number of particles and m are an increasing function of R (or, what is more significant, the

mean volume, R^3). One important characteristic is that the ratio mB_0/kT has the same dependence on 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 A). However, the largest magnetotactic microorganisms have a very high ratio of mB_0/kT, much greater than necessary to guarantee a total orientation in the geomagnetic field.

CONCLUSIONS

The results presented in this paper permit us to look at magnetotaxy in a different context.

Magnetotactic microorganisms at low concentrations can be treated as non-interacting magnetic dipoles. The average alignment of a magnetic dipole subjected to a field B_0 is given by:

$$\langle\cos\theta\rangle = \mathcal{L}\left(\frac{mB_0}{kT}\right) \qquad (3)$$

where $\mathcal{L}(x) = \coth x + 1/x$ is the Langevin function of classic paramagnetism and mB_0/kT is the ratio of the magnetic interaction energy to the thermal energy. For $x \ll 1$, $\mathcal{L} \to 0$, and so the dipoles are weakly aligned in the field, while for $x > 10$, $\mathcal{L} \sim 1$ and we have an almost complete alignment ($\langle\cos\theta\rangle \sim 1$).

Usually, it is considered that the conditions for magnetotactic orientation is given basically by $mB_0/kT > 10$ (or, the average orientation is given by $\langle\cos\theta\rangle \sim 1$) (Frankel and Blakemore, 1980). Since the migration velocity is given by $v = v_0\langle\cos\theta\rangle$, $mB_0/kT > 10$ means $v \sim v_0$. However, when the ratio mB_0/kT is of the orded of 1, the average migration velocity is about 30% of the instantaneous velocity. It has been suggested that downward directed motion is

for avoiding 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:

$$v_V = v_0 \langle \cos\theta \rangle \sin I \sim 0.3\, v_0 \sin I \qquad (4)$$

where I is the inclination of the geomagnetic field. Thus, in Rio de Janeiro (B_0 = 0.25G, $I \sim 25°$) for a microorganisms with $mB_0/kT = 1$ ($m_m = 1.6 \times 10^{-13}$ emu) we obtain:

$$v_V \sim 0.1\, v_0$$

This result indicates that even in this case there is a biological advantage (i.e., magnetotaxy can be a more efficient mechanism than chemotaxy to produce a displacement towards the bottom (Frankel, 1982). In table 1, the estimates of m result in $mB_0/kT \geq 3$ for the observed microorganisms.

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

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 period of time shorter than that between two consecutive perturbations. This means that the reversal time has to be efficient from a biological perspective, that is from the perspective of the life-span and size of the microorganism.

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., $mB_0/kT > 1$. This condition is satisfied for magnetic moments of the order of $m_m = 1.6 \times 10^{-13}$ e.m.u. in the local geomagnetic field (B_0=0.25G). The reversal time τ increases with the cube of the radius of the microorganism for a fixed value of m (eq. 1). For larger organisms, the rapid increase of τ at constant m, would make the response to magnetic stimuli inefficient. The curve a (fig. 2) shows the values of τ calculated for m_m and B_0 = 0.25G. As shown in fig. 2, we note that the reversal time (τ_0 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 sizes of organisms for which magnetotaxy by passive orientation would be efficient.

Thus, magnetotaxy 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 orient the microorganism is much smaller than the cycles of perturbations that may occur in their habitat.

APPENDIX A

In this appendix the Bean model for the movement of a magnetotactic bacteria 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 Reynold's 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 5 Gauss) 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_0, in the medium, subjected only to thermal perturbation. The average orientation is given by the classic theory of paramagnetism:

$$\langle \cos\theta \rangle = \mathcal{L}\,(mB_0/kT)$$

where $\mathcal{L}$ 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 them:

$$v = v_0 \langle \cos\theta \rangle$$

The saturation condition occurs when $mB_0/kT > 10$, which means that $v > 0.9\ v_0$.

The forces acting on the bacterium are basically the flagellar force, with 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 center-of-mass coordinate system is:

Torque from field + Viscous Drag Torque = 0

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

$$mB_0 \sin\theta - 8\pi\eta R^3 \frac{d\theta}{dt} = 0 \qquad (A.1)$$

Eq. (A.1) can be integrated exactly and we obtain:

$$\ell n\ (\tan\ \theta/2) = \frac{t}{T_0} + \ell n \tan\left(\frac{\theta_i}{2}\right) \qquad (A.2)$$

where $T_0 = 8\pi\eta R^3/mB_0$ and θ_i is the initial orientation angle.

When the field is suddenly reversed (or, what is completely equivalent, the bacterium rotates in relation to the field), $\theta_i = \pi$ and eq. (A.2) diverges.

This difficulty can be removed if we take θ_i small (i.e., $\tan\left(\frac{\theta_i}{2}\right) \sim \theta_i/2$) and equal to the average angle obtained from the Langevin function, i.e., $\theta_i \sim \left(\frac{2k}{mB_0}\right)^{1/2}$ for $mB_0 >> kT$. Then:

$$\ell n \tan\left(\frac{\theta_i}{2}\right) \sim \ell n \left(\frac{\theta_i}{2}\right) \sim \ell n \left(\frac{2kT}{mB_0}\right)^{1/2}$$

The resulting expression for the reversal time is:

$$\tau = \frac{8\pi\eta R^3}{mB_0} \ell n \left(\frac{2mB_0}{kT}\right). \qquad (A.3)$$

τ independs on the velocity v_0 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:

$$L = \int_0^{\infty} v_T\, dt = \int_0^{\infty} v_0 \sin\theta\, dt$$

Eq. A.1 gives:

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

Then

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

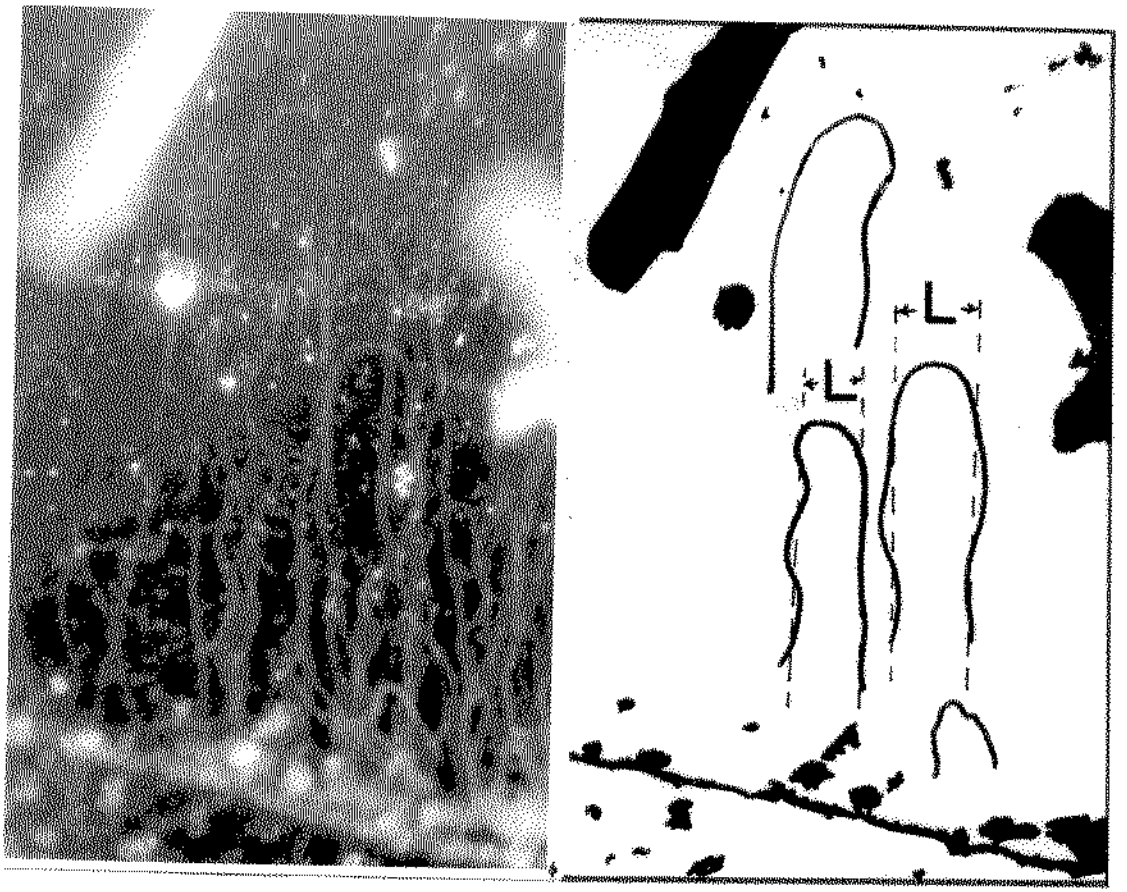
Thus, L is inversely proportional to m and B_o and is directly proportional to R^3 and v_o.

FIGURE CAPTIONS

Figure 1 : a) Dark field image from optical microscopy of the trajectories of various magnetotactic microorganisms (number 5 in table 1).
Photograph was obtained with an exposure of about 1 sec.
b) Graphic representation of the U-turn obtained from the photograph.

Figure 2 : Relation between reversal time (τ) in the local geomagnetic field (B_o = 0.25G) and volume of some magnetotactic microorganisms.
Curve a, assuming $m=1.6\times10^{-13}$ e.m.u. for all the microorganisms and τ calculated from eq. (1).
▓ - mean reversal times obtained from the estimated values of m (table 1)
\\\ - expected region for the reversal time.

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



- a -

- b -

Figure 1 .

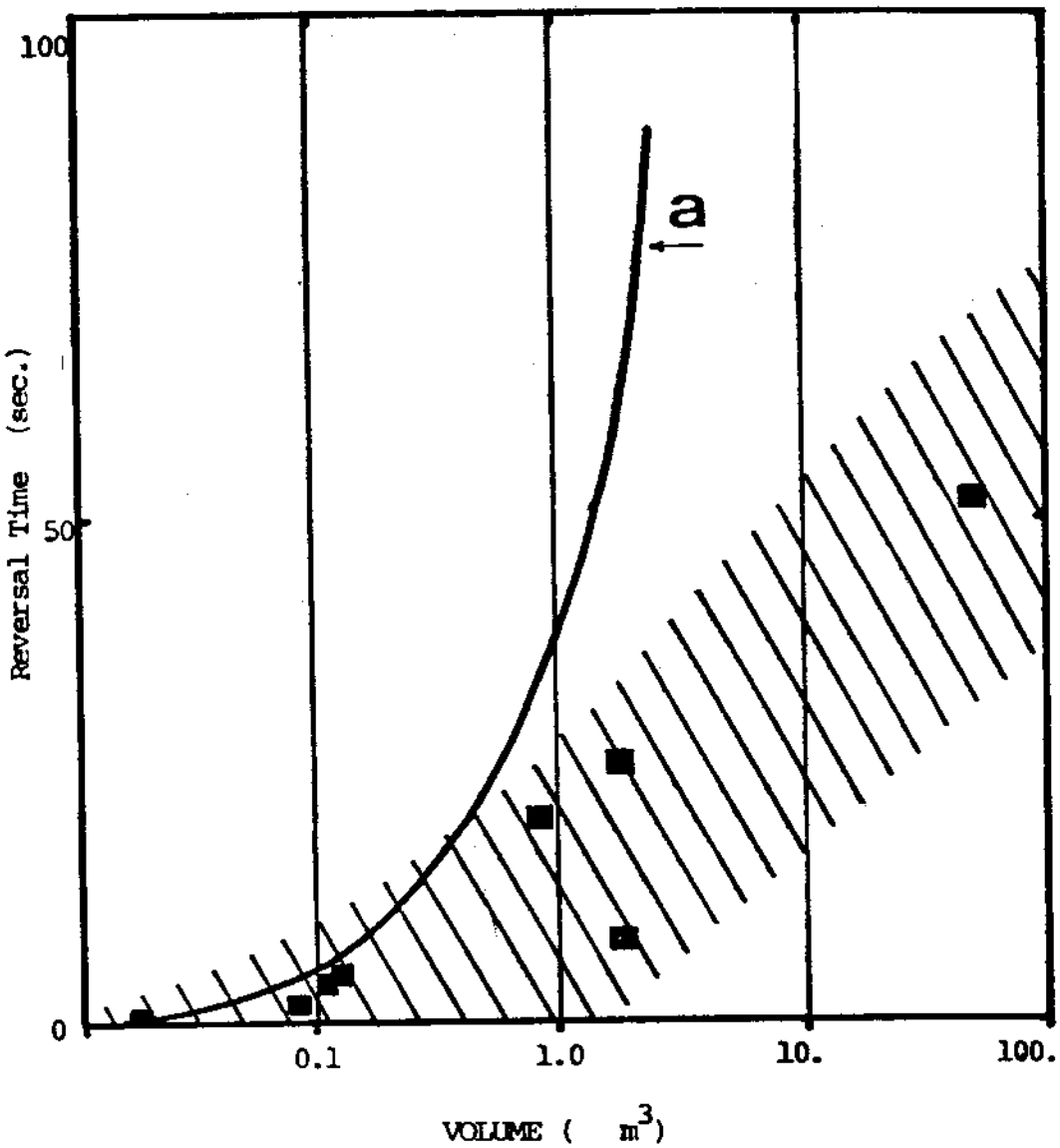


Figure 2.

TABLE 1

LOCAL	PICTURE (Bar=5μm)	R (μm)	N	B=9.3G v (μm/s)	B=9.3G τ_U(sec)	B=9.3G τ_{exp}(sec)	B=9.3G L (μm)	$m_{E.M.}$ 10^{-12} emu	m_U 10^{-12} emu	mB_o/kT	τ_o(sec)
FRESH WATER	1	.5	5	100	.08	.05	3	.5	.3	3	.5
	2	1	7	50	.3	.3	8	.7	.5	4	3.6
	3	1.5x2.5 (~2.0)	-	12	.9	1.3	8	-	1	6	20
R.DE FREITAS	4	.9	10	-	-	.09	-	1.4	-	8	1.5
	5	2.5	>1000	40	1.3	1.4	30	-	2.4	17	25
GUANABARA BAY	6	.9	10	-	-	.1	-	1.3	-	8	1.5
	7	2.5	-	70	.3	.4	11	-	8	48	8.9
	8	5x9 (~7.5)	-	30	.4	2.1	20	-	54	326	50

R - mean radius of the organism obtained in optical and/or electron microscopy
N - mean number of magnetite particles found inside the microorganism by TEM
v - mean velocity at B =9.3G (μm/sec)
L - mean diameter of the U-turn at B =9.3G
τ_{exp} -mean reversal time for the U-turn measured at B =9.3G
τ_U -reversal time calculated from eq. (1) with B =9.3G
m -estimated magnetic moment: E.M.-from electron microscopy,
U - from Bean model
mB_o/KT -ratio between the magnetic and thermal energy in local geomagnetic field (B_o=0.25G)
τ_o -reversal time for the U-turn calculated form eq. (1) with B_o=0.25G.

REFERENCES

- Blakemore R.P., 1975, Magnetotactic bacteria. Science 190:377-379
- Blakemore R.P., 1982, Magnetotactic bacteria. Ann.Rev. Microbiol. 36:217-238
- Esquivel D.M.S., Lins de Barros H.G.P., Farina M., Aragão P.H.A. and Danon J., 1983. Microorganismes magnetotactiques de la region de Rio de Janeiro. Biol. Cell. 47(2):227-234
- Farina M., Lins de Barros H.G.P., Esquivel D.M.S. and Danon J., 1983. Ultrastructure of a magnetotactic microorganism. Biol. Cell. (193)48:85-86
- Frankel R.B., 1982. Magnetotactic bacteria. Comments Mol. Cell. Biophys.1:293-310
- Frankel R.B., 1984. Magnetic guidance of microorganisms. Ann.Rev. Biophys. Bionerg.,13:85-103
- Frankel R.B. and Blakemore R.P., 1980. Navigational compass in magnetic bacteria. J.Magn.and Magn. Maters, 15-18:1562-1564
- Frankel R.B. and Blakemore R.P., 1984. Precipitation of Fe_3O_4 in magnetotactic bacteria. Phil. Trans. R. Soc. London, B304: 567-574
- Frankel R.B., Blakemore R.P. and Wolfe R.S., 1979. Magnetite in freshwater magnetotactic bacteria. Science 203:1355-1356
- Gould J.L., 1982. Ethology. The mechanisms and evolution of behavior. W.W. Norton and Co., N.Y. London
- Kalmijn Ad. J., 1978. Experimental evidence of geomagnetic orientation in elasmobranch fishes in Animal Migration, Navigation, and Homing. K. Schmidt-Koenig and W.T. Keeton (eds). Springer-Verlag. N.Y.
- Palmer J.D. (ed), 1976. An introduction to biological rhythms. Academic Press, N.Y.
- Palmer J.D., 1963. Organismic spatial orientation in very weak magnetic fields. Nature 198:1061-1062
- Rosenblatt C., Torres de Araujo F.F. and Frankel R.B., 1982a. Light scattering determination of magnetic moments of magnetotactic bacteria. J. Appl. Phys. 53:2727-2729
- Rosenblatt C., Torres de Araujo F.F. and Frankel R.B., 1982b. Birefringence determination of magnetic moments of magnetotactic bacteria. Biophys. J.40:83-85

- Schmidt-Koenig K. and Keeton W.T. (eds), 1978. Animal Migration, Navigation, and Homing. Springer-Verlag, Berlin, Heidelberg, New York.