

MAGNETOTACTIC BACTERIA

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INTRODUCTION

Many organisms have been known for a long time to sense the earth's magnetic field. Certain bacteria are also geomagnetically sensitive. The way these bacteria interact with the geomagnetic field has proven useful in thinking about ways higher organisms may also do so. In fact, the ferromagnetic detection system in bacteria has some interesting parallels in geomagnetically responsive animals. Thus, just as prokaryotes have contributed to such fields as genetics and biochemistry, they are now valuable in newly emerging fields.

Just as importantly, the magnetic property of these bacteria has permitted us to identify, collect, and study them and to begin to evaluate their niche. The magnetotactic bacteria at first appear so unique as to deserve

special taxonomic status. Indeed, all of those examined share several traits. They are Gram negative, motile (by means of flagella), microaerophilic, and aquatic, and they synthesize intracellular enveloped magnetic grains termed magnetosomes. However, despite these and perhaps other similarities, the diversity of cell morphology within the group (see Figures 1–7) and the fact that one isolate is similar to members of *Aquaspirillum* argues for rather widespread taxonomic distribution of the trait. Thus, “magnetotactic bacteria” is a descriptor similar to “gliding bacteria” or “hydrogen-oxidizing bacteria” and does not have taxonomic meaning. “Magnetotaxis” denotes cell motility directed by a magnetic field. “Taxis” implies that the magnetic field influences the swimming direction but not the absolute velocity of magnetotactic cells.

A review of current knowledge of magnetotactic bacteria might be considered premature, especially in view of the paucity of research papers available. However, my purpose is not only to critically discuss available information, but also, hopefully, to spark in some readers interest and activity in this new area of research.

The subject is a rich one that inherently crosses traditional disciplinary boundaries. Studies of magnetotactic bacteria have proven of interest and value to microbiologists, evolutionary biologists, biochemists, physicists, geochemists, paleogeologists, and animal behaviorists. These organisms also offer intriguing possibilities for biotechnology. Progress in the field has been greatly stimulated by interdisciplinary collaborations and discussions.

Discovery of Magnetotactic Bacteria

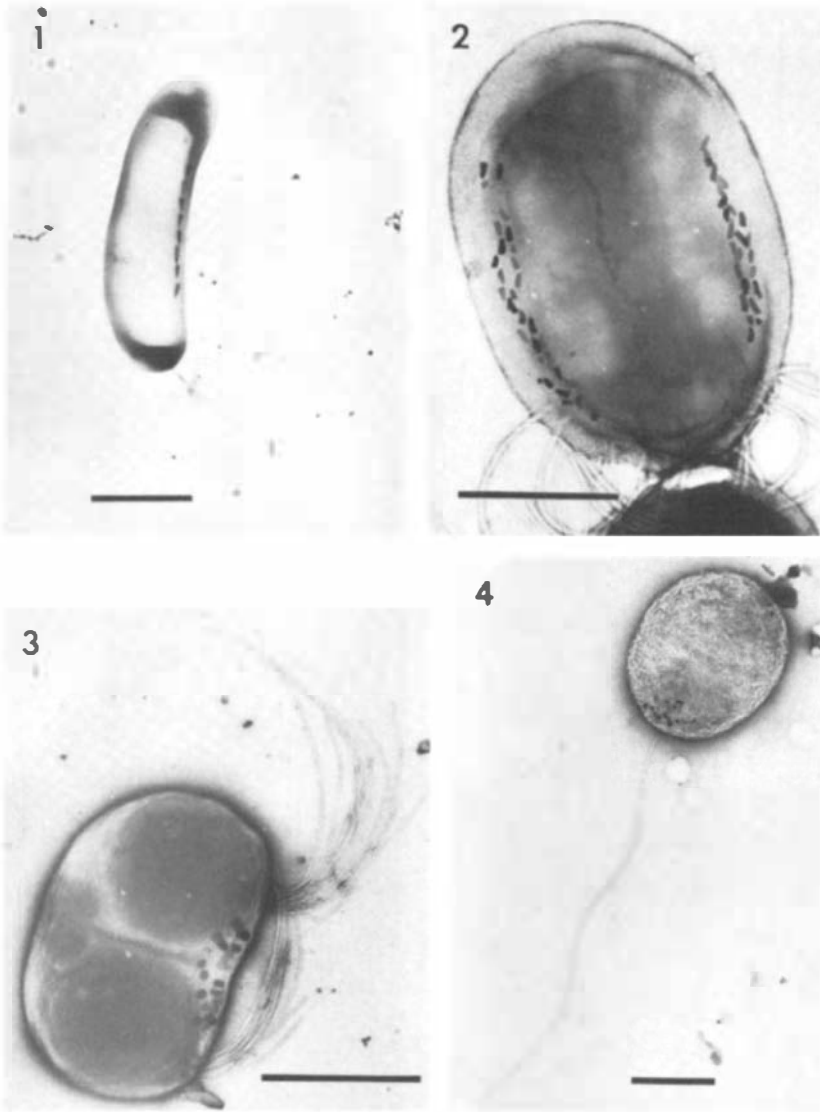
In the early 1970s as a graduate student in the laboratory of E. Canale-Parola, I was interested in the fascinating microbial populations undergoing natural enrichment within sulfide-rich mud samples stored on a laboratory shelf. Some of these he had collected from a marshy area adjacent to the Eel Pond in Woods Hole, Mass. They were of particular interest because they became enriched in the elusive *Spirochaeta plicatilis* as well as *Thiospira* and large *Beggiatoa* species (6), none of which have been cultured axenically. I casually observed that these natural enrichments also contained highly motile bacteria that migrated nearly unidirectionally across the microscope field of view. These latter microorganisms became dislodged from sediment particles in the course of making preparations for phase contrast microscopy. They then persistently swam toward and accumulated at one edge of drops of sediment transferred to depression slides. They swam in the same geographic direction even when the microscope was turned around, moved to another location, or covered with a pasteboard box. Thus, it was evident their swimming direction was influenced not by light, as I had at first supposed, but by some pervasive stimulus. That the

cells were, in fact, magnetically responsive was vividly demonstrated when a magnet was brought near the microscope. To my astonishment, the hundreds of swimming cells instantly turned and rushed away from the end of the magnet! They were always attracted by the end that also attracted the North-seeking end of a compass needle and they were repelled by its opposite end. Their swimming speed was very fast, on the order of $100 \mu\text{m/s}$, and the entire population consisting of hundreds of freely and independently swimming cells swerved in unison as the magnet was moved about nearby (4).

I wish to emphasize that this was a completely unexpected finding. A research proposal requesting support to search for geomagnetically sensitive bacteria would then have been met by peer review with exactly the same degree of attention as one submitted today proposing to detect sound production by bacteria. Yet, as far as I can determine, this simple serendipitous observation in a drop of muddy water appears to have attracted rather broad interest. For example, it seems to have unlocked thinking in at least one other field, that concerned with animal homing and migration, by unequivocally demonstrating that some organisms respond to geomagnetism by means of a ferromagnetic mechanism. It also suggests a possible origin of some of the magnetic remanence contained in the paleogeologic record.

BIOPHYSICS OF MAGNETOTAXIS IN BACTERIA

Magnetotactic bacteria each possess intracellular iron grains visible by means of electron microscopy (4, 5, 36; see Figures 1–8). Before the chemical state of the iron in these organisms was known, Kalmijn & Blakemore (27), at the suggestion of E. M. Purcell, proved that each cell contained a permanent magnetic dipole moment. Bacteria from mud enrichments were exposed to a brief ($1 \mu\text{s}$) monophasic magnetic pulse of several hundred gauss (G) delivered antiparallel to their swimming direction (27, 34). At sufficiently high pulse intensities (ca 200–800 G), the cells instantly became remagnetized by the treatment, indicating that each was intrinsically permanently magnetic. Remagnetized cells instantly made U-turns and thereafter swam opposite to their original course. Remagnetization was also effected by exposing the bacteria to Alternating Current demagnetizing fields (8, 17, 35). However, by this method a maximum of 50% of the population became repolarized. Thus, each cell behaved as though it contained a single magnetic dipole in that it could not be demagnetized. Some cells present in a population were also repolarized by being made to swim toward a finely pointed magnetic needle (8) or by encountering at close range the pole of a strong magnet. This latter observation should guide the



Figures 1-4 Magnetotactic bacteria recovered from sediments by application of a magnetic field. Cells lightly stained with uranyl acetate and viewed by TEM. Bars each represent 1 μm . (1) Curved, rod-shaped cell with single polar flagellum and single chain of tooth-shaped magnetosomes. Recovered from Little Styx River, South Island, New Zealand. (2) Ovoid, rod-shaped cell with multiple, sheathed flagella at one pole. Cells of this type were recovered from a New Hampshire bog. Each possesses one or more chains of tooth-shaped magnetosomes situated in each of three lateral cell positions. (3) Coccoid cell with two bundles of flagella

student of magnetotaxis away from the use of inhomogeneous magnetic fields (i.e. bar magnets) in favor of steady, uniform magnetic fields created with electromagnetic coil systems such as Helmholtz coil pairs (see 7).

Cell Behavior in Uniform Magnetic Fields

The local geomagnetic field is fairly uniform in configuration and intensity over most of the earth, with a value of approximately 0.5 G. It acts on the bacterial magnetic dipole in the same way it interacts with a compass needle. A cell with the axis of its magnetic moment (M) positioned at an angle (Θ) with respect to the direction of the ambient magnetic field (H) experiences a torque tending to align it in the field direction (see Figure 9A). Once aligned, no further magnetic forces are exerted on the cell. Thus, magnetotactic bacteria are not pulled northward or southward by magnetic interactions, they are merely aligned in the geomagnetic field. Indeed, killed cells do the same, also being magnetic (see Figure 9B). Magnetotaxis results from passively oriented cells swimming along magnetic field lines (see Figure 9C). Conclusive evidence of their geomagnetic sensitivity was obtained with portable Helmholtz coils operated in the field, far from the magnetic noise associated with most laboratory buildings, power transmission lines, and the like (26).

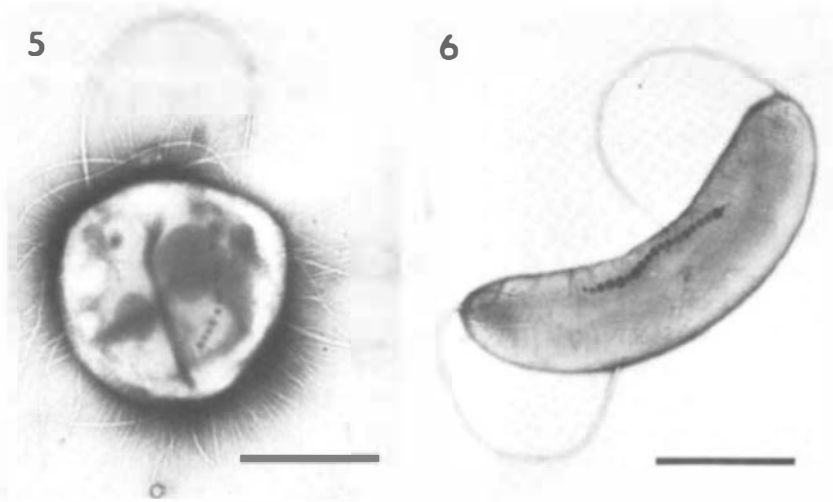
Interaction of the Cell Magnetic Dipole with the Geomagnetic Field

How well adapted are these bacteria for interacting with earth's magnetic field? Basically, to assess this we must evaluate the cell magnetic moment. Its interactive energy with the magnetic field, which tends to produce cell orientation, may then be compared with kT , the thermal forces associated with Brownian motion that tend to randomize cell orientation in water.

The average alignment of a population of noninteracting magnetic dipoles in a magnetic field is described by the Langevin function for classical paramagnetism. This relationship states that

$$\cos \Theta = L (MH/kT),$$

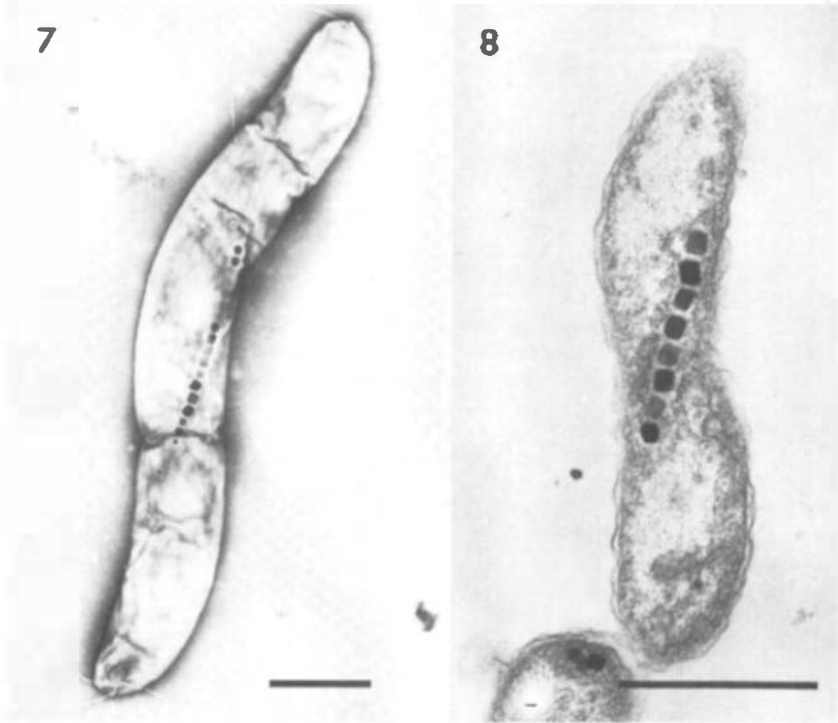
positioned at the (concave) side of the cell. Cells of this type, which are similar to those studied by Moench & Konetzka (36), were recovered from Mill Pond, Durham, New Hampshire. The cell shows a developing division plane bisecting its cluster of parallelepiped shaped magnetosomes. The large spherical dark structures contain phosphorus and potassium (see text). (4) Coccoid cell with a single polar unsheathed flagellum found in sediments of a New Hampshire bog. Parallelepiped-shaped magnetosomes are clustered at each end of cells of this type. The cell surface has the characteristically wavy appearance of the Gram-negative outer cell wall.



Figures 5 and 6 Magnetotactic bacteria with internal chains of cuboidal to octahedral magnetosomes. Cells stained lightly with uranyl acetate and viewed by TEM. Bars each represent 1 μm . (5) A coccoid cell recovered from Cedar Swamp, Woods Hole, Massachusetts. The cell has two lateral bundles of flagella and is covered with numerous, fine, pili. (6) A curved, rod-shaped cell with single bipolar flagella. Recovered from Durham, New Hampshire, water-purification plant.

where Θ is the angle between the direction of the cell magnetic moment (M) and the direction of the ambient field (H) (see Figure 9). Magnetotactic bacteria behave as noninteracting magnetic dipoles (14). Thus, their average alignment in an applied magnetic field is determined by the ratio of their interactive magnetic energy with the applied field (MH) to their thermal energy (kT). When the ratio MH/kT exceeds a value of about 10, the particles (here the bacteria) are fully aligned in the field direction (16).

At least three approaches have been used to evaluate the strength of the bacterial magnetic dipole moment. First, Frankel et al (18) showed that the magnetosomes of *Aquaspirillum magnetotacticum* strain MS-1, a magnetotactic spirillum isolated from a swamp (discussed below), consist of the magnetic iron oxide, magnetite (Fe_3O_4), also known as lodestone. Balkwill et al (2) determined the configuration, shape, size, and average number of magnetosomes per cell in this species grown under similar conditions. Each cell contained approximately 20 cuboidal-to-octahedral crystalline magnetosomes averaging 420 \AA on a side and arranged in a chain (Figures 7, 8). The particles were within the single magnetic domain size range for magnetite, which is from 400–1000 \AA (11, 29), although occasional smaller (superparamagnetic) particles were also present. It is not clear how



Figures 7 and 8 *Aquaspirillum magnetotacticum* cells showing internal chain of cuboidal magnetosomes. Bars each represent $0.5 \mu\text{m}$. (7) Negatively stained cell showing single bipolar flagella. (8) Thin-sectioned cell showing Gram-negative wall type and portion of a chain of enveloped magnetosomes. Note the transversely sectioned cell revealing proximity of the magnetosomes to the cell boundary layers.

magnetotactic bacteria limit the dimensions of their magnetosomes to the magnetically efficient single-domain size, or why these structures exist in a chain, as is frequently the case (see Figures 1, 2, 5, 6, 7). However, it is tempting to speculate that constraint is somehow invoked by the envelope surrounding each magnetite particle and by the relationship of the envelope to the remaining cell structure. Single magnetic domains constrained to lie in a chain will lie with their magnetic axes parallel, head-to-tail (24). Thus, the total magnetic energy of the magnetosome chain is the sum of the individual particle magnetic moments. From these considerations, from the volume of magnetite per cell (then estimated to average 22 particles, each cuboidal and 500 \AA on a side) and its saturation magnetization value ($480 \text{ erg/G} \cdot \text{cm}^3$), the calculated total cell magnetic energy was $1.3 \times 10^{-12} \text{ erg/G}$. In the 0.5-G geomagnetic field, the magnetic energy of such a cell ($6.6 \times 10^{-13} \text{ erg}$) was found to be 16 times greater than thermal energy, kT

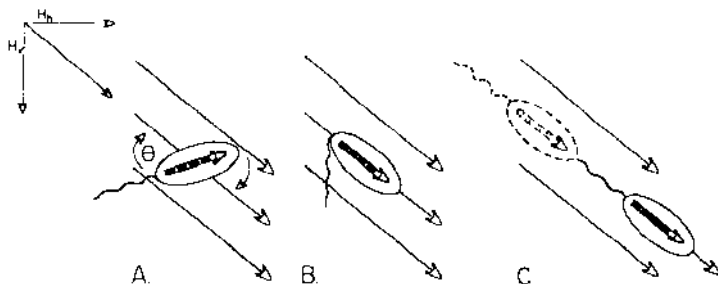


Figure 9 The Northern Hemisphere geomagnetic field is inclined downward as shown here; that of the Southern Hemisphere is inclined upward. The field direction, by convention, is the direction indicated by the North-seeking end of a magnetic compass needle. The net field (H) is comprised of a horizontal component (H_h) and a vertical component (H_v). The angle of inclination (dip) in New Hampshire is much steeper than that shown, being 73° from the horizontal. (A) An aquatic bacterium with its intrinsic magnetic dipole moment lying at an angle (θ) with respect to the field direction experiences a torque tending to align it in the uniform geomagnetic field. (B) A dead or nonmotile magnetic bacterium with magnetosomes also aligns with the field direction. (C) Motile magnetic cells are magnetotactic; they swim preferentially along magnetic field lines. Southern Hemisphere magnetotactic bacteria have opposite magnetic polarity to those in the Northern Hemisphere. Thus, both types swim down along geomagnetic field lines.

(4.1×10^{-14} erg). From these considerations, it is clear the magnetic apparatus of *A. magnetotacticum* easily accounts for the organism's ability to align in the geomagnetic field. It serves as a ferromagnetic biocompass (16).

Recently, the average magnetic moment of a population of living *A. magnetotacticum* cells was determined from light scattering (39) as well as birefringence measurements (C. Rosenblatt, F. F. Torres de Araujo, R. B. Frankel, submitted for publication). Values of $2.2 \pm 0.2 \times 10^{-13}$ and $4.3 \pm 0.5 \times 10^{-13}$ erg/G were obtained for cells in each of two spirillum suspensions. These were within 15–20% of the estimated values for the two samples based upon the total average volume of magnetite per cell as determined by electron microscopy. The method permits direct evaluation of moments for cells suspended in liquid (culture) media and is insensitive to the presence of nonmagnetic organisms, dust, or other suspended particles that would contribute to light scattering. The light scattering method also provides rapid measurement of average cell length without the need for electron microscopy.

The cell magnetic moment has also been measured in an elegant, although tedious, way (25, 41). Kalmijn et al (25, 41) tracked single magnetotactic bacteria made to run back and forth across a ruled grid on a microscope slide situated within a Helmholtz coil pair. Thus, the swimming direction and translational velocity of individual bacteria was controlled by

varying the amount and direction of current flow through the coils. A cell's migration rate (i.e. its translational velocity, not its absolute velocity) was measured as a function of the applied field strength. The migration rates of single cells plotted for various values of applied magnetic field fit a curve described by the Langevin function. Since their swimming velocity remained constant over the observation period, the average value of their angle of deviation from the field direction $\langle \cos \Theta \rangle$ was directly related to the cell migration rate. At low ambient field strengths cells deviated from the field direction more than at high field intensities. By measuring cell migration velocity for known values of H at constant temperature, values of M were obtained. The method provided values of 6.2×10^{-13} and 7.3×10^{-13} erg/G for each of two individual magnetotactic cocci obtained from a swamp-water enrichment (25). The estimated average magnetic moment of cells from the same enrichment based upon electron microscope evaluation was 8.6×10^{-13} erg/G.

The average value of the bacterial cell magnetic moment as determined by these independent methods appears to be surprisingly constant between different species. The results of Langevin analysis applied to spirilla in pure culture (16) or coccoid cells found in natural enrichments (25) indicated that magnetotactic bacteria synthesize sufficient but not excessive amounts of magnetite for their efficient interaction with the 0.5 G geomagnetic field. They are 80–90% fully aligned by a field of 0.5 G at 30°C. More than the amount of magnetite present within cells would not significantly improve the efficiency with which they interact with the geomagnetic field. Furthermore, larger cell magnetic moments than those observed might produce cell-to-cell magnetic interactions sufficiently great to override their electrostatic repulsive (zeta potential) forces, thereby causing them to clump. This may provide the selection pressure responsible for limiting the number of magnetosomes per cell in natural populations.

NATURAL ABUNDANCE, DISTRIBUTION, AND ECOLOGY OF MAGNETOTACTIC BACTERIA

Detection Methods

With the advantage of their magnetically directed motility, it has been a simple matter to detect magnetotactic bacteria in diverse natural environments (5, 8, 17, 36). The cells readily swim out of sediments when a steady field of 1–10 G is applied and can be made to accumulate in regions where they may be counted or collected for further study. Effective uniform magnetic fields are easily created with electromagnetic coils. The Helmholtz configuration (paired coils separated by a distance equal to the radius of each coil) (see 7) are preferred because of the uniformity of the resulting

magnetic fields. Serially diluting homogenized sediment samples prior to magnetic cell separation has made it possible to directly enumerate these organisms in many natural environments, as well as in laboratory experiments with altered fields.

Distribution and Abundance

Commonly, as many as 10^3 – 10^4 cells/ml of sediment slurry occur in aquatic environments sampled throughout New England. Moench (35) detected, but did not enumerate, magnetotactic bacteria in approximately 37 of 41 temperate freshwater and marine environments sampled. Only those polluted with oil, chlorine, or copper sulfate and a site containing acid mine drainage contained no magnetotactic bacteria. Other habitats in which we have sought but have not found these organisms include a limestone cavern, a thermal spring, thawed sediments collected in Antarctica, and iron-rich seeps containing abundant *Gallionella* and *Leptothrix*. Settling basins of water purification plants, sewage-treatment oxidation ponds, and natural ponds with accumulated organic sediments are particularly good collection sites. Magnetotactic bacteria are abundant in sediments collected from beneath *Typha*. Moench (35) investigated a possible relationship between *Lemna* and a freshwater magnetotactic coccus. Such relationships, if they exist at all, have been difficult to establish. However, it is not unlikely that plant-derived phenolic compounds may serve to chelate iron in a form useful to these bacteria for magnetite synthesis. Ferric quinate is a satisfactory source of chelated iron in a chemically defined culture medium for the magnetotactic spirillum (9). It is also possible that organic acids produced by plant roots serve as satisfactory sources of carbon and energy for these bacteria.

Global Distribution and Cell Magnetic Polarity

Magnetotactic bacteria are ubiquitous. They have been found in the Arctic (L. Greenfield, personal communication), in Baltic Sea sediments (R. S. Wolfe, personal communication) in South America (17), in Australia and New Zealand (8, 28), and throughout North America (5, 7, 36). Without exception, those found in the Northern Hemisphere swim predominantly northward (5, 7, 36) and those of the Southern Hemisphere swim predominantly southward (7, 8, 28). This seeming curiosity is of considerable ecological significance: It involves selective survival of cells with one direction of magnetic polarity over those of opposite polarity. What may account for this segregation of polarity types in each global hemisphere?

As mentioned previously, magnetotactic bacteria swim in unison along magnetic field lines. The earth's magnetic field is directed northward over the surface of the globe. However, the local geomagnetic field also has a

vertical component, which is directed upward in the Southern Hemisphere, decreasing to a value of zero at the geomagnetic equator, and is inclined downward in the Northern Hemisphere, with a value increasing with latitude (20). Because of this inclination, north-seeking bacteria found in the Northern Hemisphere are also directed downward there (Figure 9). South-seeking bacteria, which predominate in the Southern Hemisphere, are directed downward south of the geomagnetic equator (8, 28). Consequently, magnetotactic cells in either hemisphere are magnetically polarized relative to their principal swimming direction in such a way that they swim almost exclusively downward. This undoubtedly is an important factor determining their prevalence in sediments and absence from surface waters.

Recently, Frankel et al (17) observed that populations of magnetotactic bacteria at the geomagnetic equator consist of roughly equal numbers of cells of each magnetic polarity. Apparently cells there, when disturbed, migrate principally horizontally along the magnetic field lines, avoiding detrimental upward excursions. (The field is totally horizontal there.) However, since they exist in sediments at the equator as they do elsewhere, and not in the surface waters, it is likely that other tactic responses, to oxygen or to reduction-oxidation potential, for example, can play an important role in determining their vertical distribution in sediments.

Effects of Altered Magnetic Fields on Predominant Cell Polarity

Studies in my laboratory have been aimed at establishing the effect of altered magnetic fields on survival of magnetotactic bacteria (R. Blakemore, W. O'Brien, R. Caplan, manuscript in preparation). Loosely stoppered 125-ml vials containing 10 ml of sediment slurry and 90 ml of water from enrichment cultures containing predominantly northward and downward swimming cells were incubated within electromagnetic coils. Four coils (each consisting of 80 turns of 24-gauge magnet wire; coil length, 8 cm; coil diameter, 7.5 cm) were connected in series and were powered by an AC adapter (Archer, cat. no. 273-1435; 3-V DC; 100-mA output). With current flowing, the vertical component of the local geomagnetic field within each coil was inverted as determined with a gaussmeter. Cells in a drop of sediment from the enrichment, when placed within each coil, migrated upward and accumulated at the top of the drop when the current was on.

At approximately 4-day intervals for a period of up to 2 months, samples within each of the four coils as well as control vials placed within nonenergized coils were gently homogenized by swirling for 20 s. Direct cell counts were then made of magnetotactic bacteria present in serially diluted samples from each vial. Total population densities as well as the numbers of each polarity type present were determined by allowing the cells to migrate to

their respective edges of carefully quantitated drops placed on the stage of a dissecting microscope also within an electromagnetic coil. The total population density within control and experimental vials decreased initially from about 10^6 cells/ml and after 2 weeks it stabilized at roughly 10^4 cells/ml. The number of south-seeking bacteria in the experimental vials became detectably more numerous within 3 days after supplying current to the coils. South-seeking bacteria progressively increased in number in the experimental vials, becoming predominant after 3 weeks. The ratio of South-seeking to total magnetotactic bacteria was 0.9 to 1.0 after 5–8 weeks. This trend was reversed when current to the coils was interrupted. No corresponding changes were noted in the control population. These results confirm that the global segregation of polarity types is a response to the direction of the vertical component of the geomagnetic field. They lend strong support to the notion that in natural environments, a very real consequence of magnetotaxis in bacteria is that it directs them downward away from surface waters and toward sediments.

The direction of cell magnetic polarity could be transmitted to progeny if at cell division at least some magnetosomes were partitioned to each daughter cell (see Figure 3). Nascent magnetosomes would then have the same direction of magnetization as the inherited particles. Occasionally, a division might produce a daughter cell without magnetosomes or with superparamagnetic magnetosomes (e.g. incipient areas of crystal formation occupied by particles too small to have stable magnetic remanence). After synthesizing single-domain-sized magnetosomes, these cells would have an equal probability of being of either polarity type. Any natural population of magnetotactic bacteria includes a few cells (less than 0.5% of the total population) of the "wrong" polarity. These undoubtedly comprised the progenitors of the predominantly South-seeking population that developed in the field-reversal experiments.

Mud samples similar to those described were also incubated under zero field conditions in a mu-metal enclosure (a chamber constructed of a metal with high magnetic permeability and therefore enclosing a field-free space). After 1 month they contained equal numbers of cells of each magnetic polarity; without a vertical magnetic field there was no selection for either polarity type. However, a similar experiment indicated that oxygen may help control the vertical distribution of the bacteria. When vials were sealed to prevent continued access of air, cells of each polarity type were soon prevalent in the oxygen-depleted water above the sediments, as well as in the sediments. If the seals were then removed from some of the vials, allowing air into them, they shortly thereafter contained cells of each polarity at the sediment/water interface but not in the surface waters.

These laboratory observations collectively point up the primary role of

the vertical component of the geomagnetic field in selecting for downward-seeking cells among natural populations of magnetotactic bacteria. They also indicate the independent but consistent role of oxygen in effecting vertical migration and distribution of these bacteria in natural environments. Magnetotaxis would benefit these organisms in the absence of well-defined oxygen gradients. It might also make aerotaxis more efficient by promoting straight-line motion, thereby tending to minimize random non-vertical excursions.

ENRICHMENT, ISOLATION, AND CULTIVATION OF MAGNETOTACTIC BACTERIA

Despite considerable effort spent in several laboratories, magnetotactic bacteria have not readily yielded to isolation and axenic growth. Only one species, *Aquaspirillum magnetotacticum* strain MS-1, has been grown in pure culture (9). Consequently, much of what we know of growth conditions and metabolism of magnetotactic bacteria has been learned from studies of this organism. Many of the findings may not apply to other species.

The remarkably simple and prolific enrichment of magnetotactic organisms in stored muds (5, 36) contrasts with the difficulty in their isolation. With no special precautions for maintaining anaerobic conditions, sediments may be scooped up and placed in glass or plastic containers. When these are stored loosely covered in indirect light without being frequently mixed or disturbed, they undergo an ecological succession, the supernatant water clears, and the sediment stratifies. Orange or lighter-colored surface bands generally overlie black layers just beneath the surface sediments. Magnetotactic bacteria are abundant in the surface sediments after 2 to 5 weeks. They remain abundant for periods of up to several years with replacement of water lost by evaporation. Flowing (open system) enrichments were exploited by Moench (35), but with less success than batch (closed system) enrichments in glass jars and tubes. In many natural environments containing magnetotactic bacteria, the elements P and N are limiting for bacterial growth. Our attempts to selectively enrich for them by using PO_4^- and/or NO_3^- or NH_4^+ resulted in rapid overgrowth of other bacteria. Addition of iron salts or iron chelates did not enhance their enrichment. This is consistent with findings in my laboratory that iron concentrations greater than those commonly measured in natural environments containing these organisms (ca 1 mg of Fe/liter) do not significantly increase the magnetite yield in cells of *A. magnetotacticum*. At present, the only parameter known to selectively affect cell numbers in enrichment culture is, as discussed previously, oxygen. Presumably, their successful enrichment after a period

of several weeks corresponds to the time required to establish microaerobic zones and microhabitats compatible with their growth and long-term survival. This is interesting because it also suggests that as a group, these microaerophiles may have predominated earlier in geological history when the atmospheric oxygen content was substantially lower than at present.

After studying enrichment and survival conditions for magnetotactic bacteria in diluted mud samples, a trial isolation medium was constructed. The inoculum consisted of cells magnetically accumulated from an enrichment and washed free of most contaminating nonmagnetic forms. The medium in semisolid form supported growth of *A. magnetotacticum*, which was then cloned in microaerobic agar shake dilution tubes containing a medium of similar composition (9). A small rod-shaped magnetotactic organism also formed colonies in this medium, but it was subsequently lost upon subculture. Efforts to isolate coccoid magnetotactic bacteria abundant in marine or freshwater enrichments have met with uniformly negative results (5, 35, 36). The prolific enrichment of magnetotactic forms, coupled with the fact that they may be easily accumulated by means of a magnetic field (5, 8, 17, 36), greatly facilitates isolation efforts, however.

A. magnetotacticum cells are routinely cultured microaerobically in sealed serum vials containing liquid medium and in semisolid medium in screw-capped culture tubes (9). They do not readily or consistently initiate growth from small inocula if the dissolved oxygen is greater than approximately 3–5% of saturation (4–7 μmol of O_2 /liter at 30°C in the culture medium used). However, with headspace oxygen as high as 0.21 atm (21 kPa) in sealed, undisturbed vials of liquid medium, cells often eventually grow after a variable lag period. In this case final cell yields may be even higher than those obtained at lower initial oxygen concentrations. However, cells are not magnetotactic and do not synthesize magnetosomes under aerobic conditions (2, 9).

PHYSIOLOGY AND ULTRASTRUCTURE OF MAGNETOTACTIC BACTERIA

Aquaspirillum magnetotacticum cells are microaerophilic and chemoheterotrophic. They metabolize diverse organic acids including fumaric, tartaric, and succinic as sole sources of carbon and, presumably, energy (9). They do not grow in the absence of an organic carbon source. Escalante-Semerena et al (15) studied nitrate dissimilation by growing cells of this organism. The stoichiometry obtained (two nitrate utilized per tartrate oxidized) indicated that nitrate served as the principal electron acceptor for

cells growing microaerobically on tartrate. A trace of O_2 was also consumed (7 vs 463 μmol of nitrate utilized). Oxygen utilization increased under nitrate-limiting conditions despite lower cell yields. It probably also serves as a terminal electron acceptor in this organism under appropriate conditions. However, cells will not grow anaerobically with nitrate. Thus, oxygen may also be required as a substrate for oxygenases participating in biosynthesis of hemes or other cell constituents. A study of the electron transport system of this spirillum has revealed the presence of *b*- and *c*-type cytochromes. *a*-Type and/or *o*-type cytochromes are also present, depending upon growth conditions. Although whole cells give a negative or delayed positive oxidase test (32), a positive test has been obtained with a preparation of respiratory membrane particles (W. O'Brien, unpublished results). A magnetotactic coccoid bacterium studied by Moench was found to possess a *c*-type cytochrome (35). Products of nitrate metabolism in *A. magnetotacticum* include nitrous oxide and ammonia (9, 15). Nitrite and nitric oxide are not detectable. Nitrogen is also a presumed product since under nitrate-limited conditions nitrous oxide accumulates only transiently (3, 15). Thus, this organism is a denitrifier that can concomitantly carry out assimilatory nitrate reduction. Under conditions of low oxygen tension (less than 0.2 kPa) or with nitrate and ammonia, the organism also reduces acetylene. Acetylene reduction activity was totally inhibited by 0.2 mM NH_4^+ or NO_3^- (3).

In sealed culture vessels with the partial pressure of oxygen in excess of about 0.06 atm (6 kPa), cells of *A. magnetotacticum* grow to high final cell yields, but only after a prolonged, variable lag period. Moreover, as mentioned, they do not synthesize magnetosomes and are not magnetotactic at high oxygen values. Magnetite appears to be synthesized only in the presence of a suitable iron supply and when oxygen is limiting for growth. Neither cells of this species (32) nor magnetotactic bacteria accumulated from enrichment cultures (principally coccoid cells) (N. Blakemore, unpublished results) contain detectable catalase activity. This apparently explains their sensitivity to high oxygen at low cell densities because catalase, when supplied exogenously as a constituent of culture media, permitted aerobic growth of *A. magnetotacticum*, although cells were not magnetotactic and lacked magnetosomes (9). Cells of this organism are very sensitive to H_2O_2 , even more so than those of *Chromobacterium* spp. (N. Blakemore, unpublished results). It is well known that iron salts catalyze decomposition of H_2O_2 . Thus, extracted bacterial magnetosomes visibly decompose H_2O_2 (D. Bazylinski, unpublished results.) Magnetite synthesis, especially if proceeding under microaerobic conditions in areas adjacent to cellular sites of H_2O_2 formation, might well protect the cell from H_2O_2 toxicity by preventing

its accumulation. Nonmagnetotactic cells, even though they lack magnetosomes, also have high intracellular iron content (0.2% of cell dry weight), which might offer similar protection. Iron salts that promote aerotolerance in other microaerophiles (10, 23) do not appear to do so with this species, however (9). A role in destruction of toxic products of oxygen metabolism by high cellular Mn in *Lactobacillus plantarum* has been identified (1). In stored sediments, magnetotactic bacteria survive for long periods. In laboratory cultures of magnetotactic spirilla, which often consist of mixtures of magnetic and nonmagnetic cells, the magnetotactic cells remain actively motile and persist the longest (N. Blakemore, unpublished observation). Magnetite synthesis, or reactions leading to it, may promote long-term survival of these organisms (particularly if they occasionally experience superoptimal oxygen tensions) by helping to rid cells of toxic products of O₂ metabolism. This might be of significance in many natural settings where they occur. Due to the generally depressed availability of combined forms of nitrogen (i.e. nitrate) in these environments, cells might use more oxygen in respiration, thereby generating inhibitory amounts of peroxides or other toxic intermediates of O₂ metabolism. Superoxide dismutase activity by cells has not been measured. However, exogenously supplied bovine superoxide dismutase, known to enhance aerotolerance of the microaerophile *Campylobacter fetus* (22), had no detectable effect on cells of *Aquaspirillum magnetotacticum* (R. Blakemore, unpublished results).

Cells also appear equipped to avoid excess oxygen. They show evidence of aerotaxis in semisolid medium (9) and have been observed to localize in bands around the periphery of coverslips or air bubbles in wet mounts, as do other known aerotactic bacteria. Magnetotactic cells supplied with bog or marsh water (36) or iron quinate (9) as iron sources accumulated intracellular iron some 20,000- to 40,000-fold over its extracellular concentration. Siderophores elaborated by magnetotactic bacteria have not been detected under these conditions. Iron uptake probably occurs via non-specific (37) transport because of the relatively high extracellular iron concentration of ca 1 mg/liter. At the values of pH and Eh' at which cells grow [pH 5-7; Eh' (+)40 to (+)400 mV], dissolved iron is likely to be in the ferrous form. Chelated ferric iron would likely be released intracellularly by a reductive process (19, 37). Could iron oxidation provide an energy source for these organisms?

One method of evaluating the possible contribution of Fe₃O₄ synthesis to the overall energy budget of *A. magnetotacticum* is to consider the Gibbs free energy change for Fe₃O₄ synthesis, where

$$\Delta G = \Delta G^{\circ} + RT \ln a_{\text{products}}/a_{\text{reactants}} \quad (1)$$

Since the pathway for bacterial magnetite synthesis is not yet known, this evaluation may be made from the standard free energies of formation of reactants and products, where

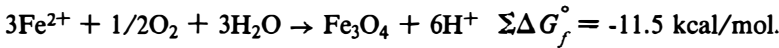
$$\Delta G^\circ = \Sigma \Delta G^\circ \text{ (formation of products)} - \Sigma \Delta G^\circ \text{ (formation of reactants)} \quad (2)$$

and

$$\Delta G^\circ = -n \mathfrak{F} \epsilon^\circ \quad (3)$$

The reactions expressing formation of Fe₃O₄ from standard state reactants and products based upon values of electrode potentials or standard free energies of formation are

Reaction	ϵ° ; volt	ΔG° kcal/mol
$6e^- + 3Fe_{(aq)}^{2+} \rightarrow 2Fe_{(s)}$	-0.4(33)	+60.9
$3Fe_{(s)} + 2O_2 \rightarrow Fe_3O_4$		-242.4(30)
$3H_2O \rightarrow 3/2O_2 + 6H^+ + 6e^-$	-1.23(33)	+170.1



Thus, the $\Sigma \Delta G_f^\circ = -11.5$ kcal/mol for the charge balanced overall reaction for magnetite synthesis. Substitution of this value into equation 1 with appropriate values for the concentration of products and reactants under conditions where magnetite is synthesized by cells (pH = 7.0; Fe²⁺ = 2.9 × 10⁻⁵ M; O₂ = 7 × 10⁻⁶ M) affords a ΔG value of -46.9 kcal/mol of magnetite produced. The negative value suggests that cells might actually derive energy from this reaction; it proceeds spontaneously. When cultured under the conditions cited, *A. magnetotacticum* attained a cell yield of approximately 30 mg (dry wt) per liter and the cells took up roughly 30% of the iron available in the medium (8 μmol of Fe/liter), thereby producing approximately 2.5 μmol of Fe₃O₄ per liter. Assuming 7–12 kcal are utilized to form 1 mol of ATP, then the energy associated with magnetite synthesis is sufficient to yield only 12 μmol of ATP per liter of culture. Assuming a value of Y_{ATP} = 10.5 μg of cell dry weight per μmol of ATP, the expected cell yield from magnetite synthesis would be 126 μg/liter. This is less than 1% of the observed cell yield. Under laboratory growth conditions, magnetite synthesis by oxidation of ferrous iron would produce an insignificant fraction of the cells' total energy budget. This supports the conclusion arrived at by Moench & Konetzka (36) that magnetite within magnetotactic bacteria is not merely an accumulated by-product produced during energy

transformation involving iron oxidation. However, studies in progress in my laboratory are directed at the possibility that cells may use Fe^{3+} during heme synthesis (12) or as a terminal electron acceptor under appropriate conditions.

The process of magnetite synthesis by bacteria is currently under study (W. O'Brien, R. Frankel, R. Blakemore, manuscript in preparation). It has as an interesting precedent in the studies of magnetite biomineralization in the marine chiton (Polyplacophora). The radular teeth of this mollusk are arranged in long parallel rows and undergo a progressive sequential mineralization that leads to a surface coating of magnetite on the most anterior denticles (31). The stages in magnetite formation in chitons include accumulation of ferritin within an organic unmineralized micellar framework, and transformation of ferrihydrite ($5 \text{Fe}_2\text{O}_3 \cdot 9\text{H}_2\text{O}$) in the ferritin cores to magnetite. The latter stage involves reduction of one third of the ferric iron to ferrous iron, oxygen removal, dehydration, and finally a change in crystal form from hexagonal close packing to cubic inverse spinel (29, 42, 43). Mössbauer spectroscopic studies of *A. magnetotacticum* have resulted in some interesting parallels. Mössbauer spectra of this organism contain, in addition to the typical six-line spectrum of magnetite, a central pair of absorption lines that correspond to those obtained from ferritin (18). Until recently, however, ferritin was not known to be present in prokaryotic cells (21, 40). Consequently, its role(s) and distribution in these organisms have not been widely studied. Recent studies of ^{57}Fe -enriched magnetotactic bacteria at low temperature have confirmed the ferritin-like behavior of this material, suggesting that it may also be a magnetite precursor in the bacteria. If this process of magnetic synthesis is common to both organisms, the magnetosome sheath may then correspond functionally and structurally to the micellar boundary layer (29, 43) observed in the mineralizing chiton radular denticle.

The magnetotactic bacteria examined to date all appear to be Gram negative. They stain appropriately and most have the typical wavy outer cell wall characteristic of this group (2; see also Figures 3, 4, 6, 8). Many species accumulated from sediments appear quite different morphologically from described species of bacteria. One ultrastructural feature common to all, however, is the presence of intracellular magnetosomes. In those species in which they have been studied, the magnetosomes were shown by means of Mössbauer spectroscopy (18), X-ray diffraction (14), or electron optical analysis (44) to consist of magnetite, Fe_3O_4 . However, the magnetosome composition of all species is not known and conceivably other magnetic minerals such as other oxides of iron or cubic iron sulfides similar to greigite (13) might be present in some. Extracted magnetosomes appear either to be contaminated with cell substances or contain substances in addition

to Fe_3O_4 . Despite different extraction procedures used in two separate studies, the magnetosome fraction consisted of from 14 (14) to 79% (44) magnetite by weight. Mössbauer spectroscopic measurements of extracted magnetosomes of *A. magnetotacticum* have revealed the presence of high-spin Fe^{3+} in oxygen coordination, which, as mentioned previously, behaves at low temperature as ferritin. This organism's magnetosomes, unlike those of a magnetotactic coccus studied by Towe & Moench (44), contain no detectable titanium and exist as cubic-to-octahedral grains (42 nm on a side) rather than hexagonal prisms (parallelepipeds approximately 100 X 62 nm). Magnetosomes consistently exist in chains situated at the cell boundary in the spirilla (2; Figure 8), although as indicated by Figures 3 and 4, they may occur in clumped or scattered arrangements in other bacteria (see also 35, 44). At cell division, the magnetosomes appear to be more or less equally partitioned to each daughter cell by the cell division plane (7; Figure 3).

Recent elemental mapping studies of magnetotactic bacteria using energy-dispersive X-ray analysis have indicated the presence of substantial amounts of phosphorus within cells. This appears to be a consistent feature of cell composition in the several species examined. However, in the coccoid types (Figures 3, 5), the cell phosphorus is localized in large spherical bodies along with potassium (R. Blakemore, unpublished results). Iron, sulfur, calcium, and magnesium also present within these bacteria in substantial amounts are absent from the phosphate bodies.

All magnetotactic bacteria examined to date possess flagella, usually positioned at one side of the cell or at the ends of cells. Some cells have bipolar flagella as does *A. magnetotacticum*. Bipolarly flagellated organisms swim in either direction along magnetic field lines. Presumably, the direction chosen under natural conditions is determined by factors such as concentration gradients of oxygen. Other magnetotactic bacteria appear to be more or less unidirectional in their motility: swimming persistently forward with a characteristic wobbling motion. These do not show the run-and-tumble motility pattern characteristic of many chemotactic flagellated bacteria. However, other tactic responses may override magnetotaxis, because, as mentioned previously, even these magnetotactic bacteria may be found in oxygen-depleted surface waters.

An interesting feature of the ultrastructure of these bacteria is the presence of surface appendages (pili) often observed on coccoid forms (Figure 5). Other forms (the spirilla) produce extracellular polysaccharide material that stains with ruthenium red (2). These aspects of the outer surfaces of cells are consistent with microscope observations that these organisms adhere to sediment particles. Perhaps it is only when disturbed or dislodged from surfaces to which they may attach themselves that magnetotaxis may affect their distribution and location.

CONCLUDING REMARKS

As expected of a newly described phenomenon, research opportunities with magnetotactic bacteria abound. This is a fascinating, diverse group of organisms with a single species in pure culture. The cells sequester iron, accumulating it intracellularly to a significant fraction (2–4%) of their dry weight as a magnetic iron oxide containing two thirds of its iron in the ferric form and one third in the ferrous state. Because of the unique size, shape, and configuration of its magnetic grains, a single cell cannot be demagnetized, and its magnetic moment interacts effectively with the geomagnetic field so as to produce a torque tending to align the organism with geomagnetic field lines. It is interesting that each cell's magnetic axis coincides well enough with its axis of motility to effect rather direct swimming along magnetic field lines. An ecological consequence is that in natural environments when a vertical component of the ambient magnetic field is present, cells' direction of magnetic polarity relative to their principal swimming direction guides them generally downward toward sediments. This also keeps them away from more aerobic surface waters. The possibility that bacterial intracellular magnetite is a product of some physiological process common to all magnetotactic forms is under investigation. An understanding of the manner in which magnetite is formed by these organisms will contribute to an appreciation of their role(s) in biogeochemical cycling. These bacteria illustrate how the environmental cycles of nitrogen, carbon, and iron, for instance, may be coupled together by microorganisms.

The deposition of biogenic magnetic fine particles in sediments due to death and lysis of bacteria should have obvious interest among paleomagnetists. A study to investigate the possible contribution of bacterial magnetite to magnetic remanence of sulfide-rich salt marsh sediments was unsuccessful (13), but that does not rule out the possibility that the fine-grain magnetic iron sulfides detected may be of microbial origin. They appeared similar to particles found within magnetotactic cells shown in Figures 5 and 6, which are of unknown composition. The possible involvement of bacteria is strengthened by recent findings (W. Ghiorse, personal communication) of particles with the shape and dimensions of bacterial magnetite grains both within and outside of cells found in sediments collected in an area of manganese nodule formation in Oneida Lake of New York State (see also 5).

Recently, large numbers of magnetic cells of *Tetrahymena* were successfully produced by feeding these protozoans intact cells of *Aquaspirillum magnetotacticum* (J. Rifkin, unpublished data). We have also observed magnetically responsive protozoans grazing on accumulated magnetotactic bacteria in enrichment cultures. Although these latter observations may provide a useful means of manipulating these organisms, they bear little

relation to their natural activities. One may also ponder the ecological consequences and biological adaptiveness of magnetism in at least two other instances recently reported among microorganisms. A non-magnetotactic, magnetic, aerobic, motile, Gram-negative bacterium, strain 1173, was isolated by Rades-Rohkohl et al from a weathered rock surface (38). Neither it, nor several other iron-accumulating bacterial strains, cells of which were attracted to the poles of magnets placed near cultures, possessed magnetosomes, however.

Finally, a magnetotactic green alga belonging to the genus *Chlamydomonas* was discovered (H. Lins de Barros, et al, unpublished information) in a polluted brackish coastal lagoon of Brazil. These eukaryotic cells contain a permanent magnetic dipole moment estimated to be ten times greater than those of magnetotactic bacterial cells. The direction of their cell polarity is such that they are steered generally downward in the geomagnetic field. The presence of magnetosomes has not been substantiated, but it is inferred from demagnetization studies. The ecological (behavioral) significance of magnetotaxis in this alga is not clear. Above a certain light threshold, the organisms are negatively phototactic. Possibly cells with their direction of magnetic polarity orienting them so they swim up and localize in the highly illuminated surface waters might be selected against, whereas those appropriately magnetized to swim down would be consistently favored.

These recent findings all provide about as much wonder as they do information concerning the adaptive significance of magnetism in diverse microorganisms. They suggest that rewarding research, in addition to providing newly isolated strains now needed for comparative studies, will address the physiological implications of magnetite formation in microorganisms, studies that will require genetic and enzymatic techniques of analysis.

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