References and Notes

1. C. Enroth-Cugell and J. Robson, *J. Physiol. (London)* **187**, 517 (1966); B. G. Cleland, M. W. Dubin, W. R. Levick, *ibid.* **217**, 473 (1977); S. Hochstein and R. M. Shapley, *ibid.* **262**, 237 (1976); *ibid.*, p. 265; K.-P. Hoffmann, J. Stone, S. M. Sherman, *J. Neurophysiol.* **35**, 518 (1972).
2. K. E. Kratz, S. V. Webb, S. M. Sherman, *Vision Res.* **18**, 1261 (1978).
3. Another discrete subsystem of the cat's retinogeniculate pathway, the W system, which presumably passes only through the C laminae of the cat's dorsal lateral geniculate [P. D. Wilson, M. H. Rowe, J. Stone, *J. Neurophysiol.* **39**, 1193 (1976); M. H. Rowe and J. Stone, *Brain Behav. Evol.* **14**, 185 (1976)], is not considered since this study is limited to laminae A and A1.
4. S. M. Sherman, K.-P. Hoffman, J. Stone, *J. Neurophysiol.* **35**, 532 (1972).
5. S. M. Sherman, J. R. Wilson, R. W. Guillery, *Brain Res.* **100**, 44 (1975); S. LeVay and D. Ferster, *J. Comp. Neurol.* **172**, 563 (1977); L. J. Garey and C. Blakemore, *Exp. Brain Res.* **28**, 259 (1977).
6. K. E. Kratz, S. V. Webb, S. M. Sherman, *J. Comp. Neurol.* **181**, 615 (1978).
7. S. M. Sherman and J. Stone, *Brain Res.* **60**, 224 (1973).
8. M. Loop and S. M. Sherman, *ibid.* **128**, 329 (1977).
9. These data were obtained from normally reared cats in our laboratory according to the identical techniques used for the dark-reared cats (*4*).
10. The cells classified as normal in the cats reared in the dark, in addition to having qualitatively normal receptive field properties, also responded to shock of the optic chiasm within an expected latency range. From our sample of normal neurons, the mean ($\bar{X}$) latency ± standard deviation (S.D.) for Y cells was 1.3 ± 0.2 msec, and for X cells was 2.0 ± 0.2 msec. In the cats reared in the dark, the latency for Y cells was 1.3 ± 0.2 msec, for X cells it was 2.1 ± 0.3 msec, and for the 21 abnormal cells that responded to optic chiasm shock, the latencies included the entire range seen normally (range, 1.0 to 2.5 msec; $\bar{X}$ ± S.D., 1.8 ± 0.4 msec).
11. The 30 abnormal cells encountered in the four cats reared in the dark can be further subdivided into the following categories. (i) Eight cells appeared to be Y cells on the basis both of latency to electrical stimulation to the optic chiasm and of responses to moving stimuli, but they had abnormal spatial summation properties. That is, they displayed a clear "null position" (zero response) to a stimulus that bisected the receptive field along a line separating zones flashing on and off 180° out of phase with one another (*2*). This can be taken as evidence for linear spatial summation. Such cells, which otherwise had properties of Y cells, were never seen in normal cats. (ii) The opposite was seen in four neurons that appeared to be normal X cells except that no null position could be found. Such cells are occasionally seen in normal cats, however, and could be the result of artifacts such as eye movements (*2*). To consider these as normal X cells would in no way affect our conclusions; instead, the inability to record Y cells would seem more dramatic. (iii) Four cells with Y-like receptive fields had abnormally long latencies to optic chiasm shock (> 1.8 msec), and such cells were never seen in normal cats. (iv) Eight cells responded poorly and six cells did not respond to visual stimuli. Such abnormal cells as described here were also seen in other studies of deprived Y cells [T. T. Norton, V. A. Casagrande, S. M. Sherman, *Science* **197**, 784 (1977); (*6*)].
12. R. W. Guillery, *J. Comp. Neurol.* **144**, 117 (1972); S. M. Sherman, R. W. Guillery, J. H. Kaas, K. J. Sanderson, *ibid.* **158**, 1 (1974).
13. K. J. Sanderson, *ibid.* **153**, 239 (1974).
14. For details of these measurement techniques, see Kalil (*15*).
15. R. Kalil, *J. Comp. Neurol.* **178**, 451 (1978).
16. T. N. Wiesel and D. H. Hubel, *J. Neurophysiol.* **26**, 978 (1963); R. W. Guillery and D. J. Stelzner, *J. Comp. Neurol.* **139**, 413 (1970); T. L. Hickey *et al.*, *ibid.* **172**, 265 (1977).
17. R. Kalil, *J. Comp. Neurol.* **178**, 469 (1978).

18. Supported by NIH grant EY 01565 and NIH research career development award EY 00020 (S.M.S.), NIH grant EY 01331 (R.K.), and NIH postdoctoral fellowship EY 05077 (K.E.K.).

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31 July 1978; revised 6 October 1978

Magnetite in Freshwater Magnetotactic Bacteria

Abstract. *A previously undescribed magnetotactic spirillum isolated from a freshwater swamp was mass cultured in the magnetic as well as the nonmagnetic state in chemically defined culture media. Results of Mössbauer spectroscopic analysis applied to whole cells identifies magnetite as a constituent of these magnetic bacteria.*

Iron-containing bacteria from diverse aquatic environments which orient and swim in a preferred direction in weak (0.1 gauss) magnetic fields (magnetotaxis) have been described (*1*). Cellular iron is localized in crystals (100 by 150 nm) within these bacteria. Kalmijn and Blakemore (*2*) demonstrated geomagnetic orientation by similar bacteria in salt-marsh sediments. These workers subsequently obtained evidence through cell remagnetization studies that the bacteria exhibited properties of single domain ferromagnets (*3*). Thus, the directed swimming response of magnetic bacteria to geomagnetism is a direct one, clearly different from electromagnetic induction exhibited by elasmobranch fishes (*4*).

Definitive studies of the chemical nature of iron in magnetic bacteria have not been possible because the organisms have not been available in pure culture. Recently, Blakemore (*5*) isolated a freshwater magnetotactic bacterium. In this report, we describe the results of Mössbauer spectroscopic analyses of magnetic and nonmagnetic whole cells of this isolate cultured in chemically defined media.

The organism was an unclassified magnetotactic spirillum (Fig. 1) designated strain MS-1. It was isolated from sediments of Cedar Swamp, Woods Hole, Massachusetts, and appears to be a new bacterial species by criteria separate from its magnetic properties. Characterization, taxonomy, and details of culturing this organism have been studied (*6*). Cells of the organism were cultured under microaerobic conditions (the O_2 atmosphere over the cultures was initially 6 to 7 μM) in a liquid medium containing filtered bog water with succinic acid and sodium nitrate as the principal sources of carbon and nitrogen, respectively. Subsequently, a chemically defined medium lacking bog water was employed. Iron was supplied in this latter culture medium to a final total iron concentration of 1.6 mg/liter, as ferric sulfate and ferric quinate. Results of atomic absorption spectrophotometric analyses indicated that magnetic cells contained 1.5 percent of their dry weight as iron. The cells contained an average of 22 intracellular crystals, each approximately 50 nm on a side (Fig. 1).

After prolonged culture of strain MS-1 in a medium with less iron, cells grew nonmagnetically. They did not align with stationary external magnetic fields or rotate in response to reversal of the ambient field. From such a culture, a homogeneously nonmagnetic population of cells was obtained by standard microbiological cloning procedures. Nonmagnetic cells lacked intracellular crystals present in magnetic cells and contained less than one-tenth the amount of iron of magnetic cells. In other respects, the two types were similar. Nonmagnetic cells were maintained in a chemically defined medium identical to that used for magnetic cells, except that ferric quinate was deleted. The total iron content of this medium was 3.6 μM.

Cells were mass cultured at 30°C in glass carboys having a 10-liter capacity. They were harvested by continuous flow centrifugation (15,000 rev/min) at 10°C. Cell yields were (wet weight) 0.2 to 0.5 g per liter. Harvested cells were washed three times in distilled water and lyophilized. They were not exposed to magnetic fields stronger than those normally associated with general laboratory conditions (such as a-c motors, pumps, and electrical lines) during growth, harvest, or preparation for analyses.

Mössbauer spectra at room temperature were obtained with 350-mg samples of freeze-dried cells grown under various conditions. Cells analyzed included (i) magnetic cells grown in medium containing bog water, (ii) magnetic cells grown in chemically defined medium containing 29 μM iron, and (iii) nonmagnetic cells grown in chemically defined medium containing 3.6 μM iron.

No discernible γ-ray absorption greater than 0.2 percent was observed in nonmagnetic cells (Fig. 2a). The Mössbauer spectrum of magnetic cells grown in medium containing bog water (data not shown) was identical to that of magnetic cells cultured in chemically defined medium (Fig. 2b). The spectrum of Fig. 2b can be characterized as being due primarily to iron in magnetite (*7*). There are, however, two significant differences between the spectrum in Fig. 2b and the spectrum of stoichiometric magnetite (Fig. 2c). These are (i) an extra absorption area close to $v = 0$ (Fig. 2b), which

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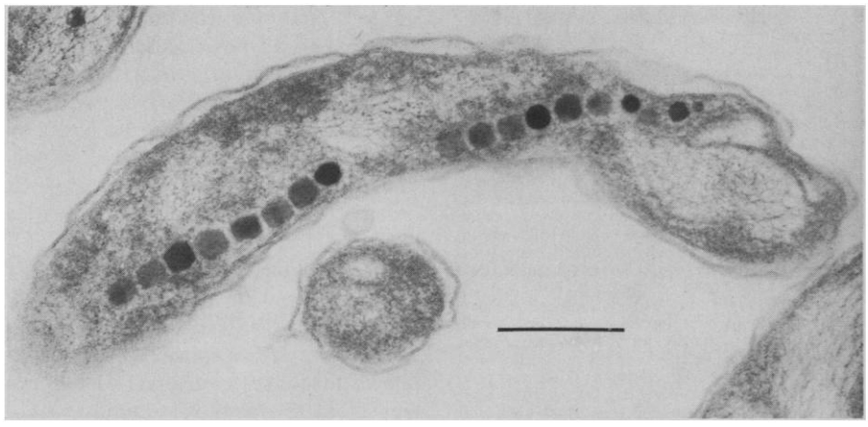

Fig. 1. Electron micrograph of thin-sectioned magnetic cells of strain MS-1. Chains of crystals are present within the cell; bar is 250 nm.

appears to be that of a quadrupole doublet and (ii) a ratio of intensities of the two lowest velocity lines (Fig. 2b) which is closer to 1:1 than 1:2 as in the spectrum of stoichiometric magnetite (Fig. 2c). The spectrum of Fe_3O_4 (magnetite) at room temperature is a superposition of two six-line spectra. One corresponds to Fe^{3+} in tetrahedral sites and the other to Fe^{3+} and Fe^{2+} connected by rapid electron exchange in octahedral sites. Introduction of vacancies in the octahedral sublattice (*8*) or γ-Fe_2O_3 on the surface of the particle (*9*) will cause the relative intensities of the two subspectra to change, with consequent changes in the relative intensities of the two lowest velocity lines. Hence, the iron-containing material in the freshwater bacteria grown in bog water medium, or in chemically defined medium, can be described as ferrimagnetic Fe_3O_4, with either ~ 4 percent vacancies in the octahedral sublattice or with a small (several percent) admixture of a γ-Fe_2O_3 phase. Mössbauer spectra obtained at low temperature and in external magnetic fields are consistent with the identification of the iron-containing material as magnetite (data not shown).

The material producing the extra quadrupole doublet cannot be precisely identified as yet but from the isomer shift it appears to be Fe^{3+} with oxygen coordination. One possibility is magnetite which is not of sufficient size to be fully magnetized single domain particles, that is, superparamagnetic magnetite (*10*). Another possibility is an iron-storage protein such as ferritin, which also produces a quadrupole doublet at room temperature (*11*). However, ferritin itself has not been identified with prokaryotic cells. Finally, it may be another iron-storage compound (*12*) or an uncharacterized iron-containing compound in which the density of iron is too low to be magnetically ordered at room temperature.

Magnetite crystals with dimensions of those present in strain MS-1 (50 nm) are within the single domain size range according to the calculations of Butler and Banerjee (*13*) and the measurements of Dunlop (*14*). Each cell has a calculated magnetic moment, $M = 1.3 \times 10^{-12}$ electromagnetic unit, sufficient to produce alignment in the geomagnetic field, $H = 0.5$ gauss, at ambient temperatures ($MH = 6.6 \times 10^{-13}$ erg, $k_BT = 4.1 \times 10^{-14}$ erg, where k_BT is the thermal energy at $T = 300$ K). Thus, the magnetite in these cells constitutes a biomagnetic compass, in confirmation of the original hypothesis of Kalmijn and Blakemore (*3*).

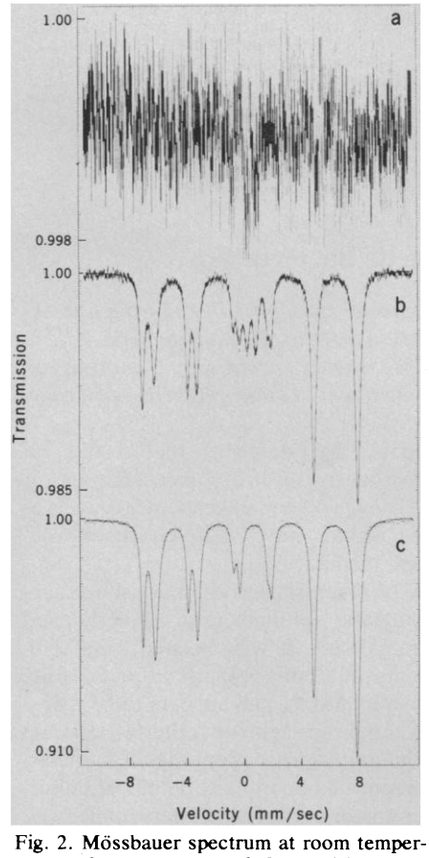

Fig. 2. Mössbauer spectrum at room temperature of (a) 350 mg of freeze-dried nonmagnetic cells of strain MS-1 grown in chemically defined medium, (b) 350 mg of freeze-dried magnetic cells of strain MS-1 grown in chemically defined medium, and (c) stoichiometric magnetite (Fe_3O_4), obtained by crushing a freshly prepared interior slice of a magnetite single crystal. Solid lines through the spectra tracings are theoretical least-squares fits to the data, based on Lorentzian line shapes.

Magnetite is present in the radular cappings of chitons (*15*), in the abdomens of live bees (*16*), and the skulls of pigeons (*17*). Except for magnetic bacteria, however, it has not been demonstrated that the presence of magnetite in living organisms bears any relation to their orientational responses to external magnetic fields. Thus, the bacteria provide a basis for considering the intriguing possibility that magnetite may also be involved in the orientational responses of some eukaryotic organisms to geomagnetism.

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References and Notes

1. R. P. Blakemore, *Science* **190**, 377 (1975).
2. A. J. Kalmijn and R. P. Blakemore, *Proc. Int. Union Physiol. Sci.* **13**, 364 (1977).
3. _____, in *Animal Migration, Navigation and Homing*, K. Schmidt-Koenig and W. T. Keeton, Eds. (Springer-Verlag, New York, 1978), p. 344; see also *Sci. Am.* **238**, 72 (March 1978).
4. A. J. Kalmijn, in *Animal Migration, Navigation, and Homing*, K. Schmidt-Koenig and W. T. Keeton, Eds. (Springer-Verlag, New York, 1978), p. 347.
5. R. P. Blakemore, while working in the laboratory of R. S. Wolfe.
6. _____, D. Maratea, R. S. Wolfe, in preparation.
7. W. Kundig and R. S. Hargrove, *Solid State Commun.* **7**, 223 (1969).
8. V. P. Romanov and V. D. Chercherskii, *Sov. Phys.-Solid State* **12**, 1474 (1970).
9. H. Topsoe, J. A. Dumesic, M. Boudart, *J. Phys. (Paris)* **12**, C6-411 (1974).
10. T. K. McNab, R. A. Fox, A. J. F. Boyle, *J. Appl. Phys.* **39**, 5703 (1968).
11. F. A. Fishbach, D. W. Gregory, P. M. Harrison, T. G. Hay, J. M. Williams, *J. Ultrastruct. Res.* **37**, 495 (1971).
12. H. A. Lowenstam and G. R. Rossman, *Chem. Geol.* **15**, 15 (1975).
13. R. F. Butler and S. K. Banerjee, *J. Geophys. Res.* **80**, 4049 (1973).
14. D. J. Dunlop, *ibid.* **78**, 1780 (1973).
15. H. A. Lowenstam, *Geol. Soc. Am. Bull.* **73**, 435 (1962).
16. J. L. Gould, J. L. Kirschvink, K. S. Deffeyes, *Science* **201**, 1026 (1978).
17. C. Walcott, personal communication.

18. We thank D. R. Balkwill for electron microscopy; N. A. Blakemore and D. Maratea for technical contributions; and A. J. Kalmijn for inspiration and support. Supported by NSF grants to the Francis Bitter National Magnet Laboratory and by NSF grant PCM 77-12175.

26 October 1978; revised 27 November 1978