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SHORT COMMUNICATION
FERROMAGNETISM IN TWO MOUSE TUMOURS

BY JOSEPH L. KIRSCHVINK

Division of Geological and Planetary Sciences, The California Institute of Technology, Pasadena, California

FRANK L. TABRAH

Cancer Center of Hawaii, School of Medicine, University of Hawaii, Honolulu, Hawaii

AND STANLEY BATKIN

Cancer Center of Hawaii, School of Medicine, University of Hawaii, Honolulu, Hawaii

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A variety of living organisms has been found recently that are biochemically able to precipitate the ferromagnetic mineral magnetite (Fe_3O_4). Originally discovered in the radular teeth of a primitive marine mollusc (Lowenstam, 1962), magnetite has since been reported in bacteria (Frankel, Blakemore & Wolfe, 1979), arthropods (Gould, Kirschvink & Deffeyes, 1978), and vertebrates (Walcott, Gould & Kirschvink, 1979; Zoeger, Dunn & Fuller, 1981; Walker & Dizon, 1981). Although the presence and biological origin of this material are clear, very little is yet known about the distribution or metabolic function of ferromagnetic minerals in vertebrate tissue. Magnetic remanence, which uniquely indicates the presence of ferromagnetic particles, has been previously detected in localized areas associated with the dura membranes of homing pigeons (Walcott *et al.* 1979) and dolphins (Zoeger *et al.* 1981), in pigeon neck muscles (Presti & Pettigrew, 1980), in the mid-brain of monkeys, and in human adrenal glands (Kirschvink, 1981). We report here the first discovery of anomalously high concentrations of ferromagnetic material in two strains of neoplasms, YC-8 lymphoma and Lewis lung tumour, as well as the apparent absence of such material in three human carcinomas (gastric, colon and renal).

Our previous studies revealed biological effects of non-ionizing radiation on tumour cells (Batkin & Tabrah, 1977). In a trial study to attempt to enhance this effect, we added paramagnetic spin-labelled compounds to the cells *in vitro*. Cell changes were observed after the addition of the paramagnetic compounds alone (Batkin, Tabrah & Misconi, 1980) and after further exposure to altered magnetic fields. Reports of ferritin-bearing tumours (Gropp, Havemann & Lehmann, 1978; Hann *et al.* 1979; Kew *et al.* 1978) and of magnetite in varied biological specimens led us to analyse selected tumours in our laboratory for the presence of ferromagnetic material.

Cells of Lewis lung tumour and YC-8 lymphoma were maintained by repeated subcutaneous transplant in female C57BL/6 and BALB/c mice. Experimental tumours were excised from stock animals using non-magnetic glass microtome knives

Table 1. *Ferromagnetic remanence in mouse tumours*

Tumour tissue	sIRM (pT)	Sample volume (ml, ± 10%)	Tumour cells per sample (± 10%)	Average remanence per cell ([J/T]/cell)
YC-8 lymphoma				
Sample A	840	0·44	$7·8 \times 10^7$	$4·7 \times 10^{-17}$
Sample B	1290	0·18	$3·1 \times 10^7$	$7·2 \times 10^{-17}$
Lewis lung				
Sample A	750	1·71	$10·9 \times 10^7$	$11·8 \times 10^{-17}$
Sample B	620	0·86	$5·5 \times 10^7$	$9·7 \times 10^{-17}$

The induced magnetization, termed a saturation Isothermal Remanent Magnetization (sIRM), is the magnetic moment measured on the superconducting magnetometer in a zero field divided by the sample volume. Although the saturation magnetization for magnetite is 48 milli-Tesla (mT), the saturation remanence for a dispersion of single-domain crystals is half of this, or 24 mT. An electromagnetic unit (e.m.u.) is 1 Gauss.cm^3 or 10^{-3} J/T.

Note: The following human tissues were found to have no measurable remanence ($< 10^{-10}$ J/T); normal stomach (control), gastric carcinoma, colon carcinoma, and renal carcinoma. Control samples of muscle and connective tissue from normal female C57BL/6 and BALB/c mice displayed only weak saturation remanences of 32 and 45 pT respectively.

on the 13th day following transplant. Control samples of muscle and connective tissue from both strains of mice were also taken for comparison. In addition, three human carcinomas (gastric, colon and renal) were obtained after routine surgery in Honolulu. All tissue samples for magnetic analysis were first thoroughly cleaned using ultrasonic treatment in glass-distilled water. Using glass knives to avoid possible contamination from metal instruments, each tumour was subdivided into two or more subfractions of appropriate size for the SQUID magnetometer. All samples were frozen prior to and during the magnetic analysis.

Magnetic measurements on these tissue samples were performed on a super-conducting rock magnetometer of the type described by Goree & Fuller (1976), which was housed in a magnetically shielded mu-metal room. To reduce the chance of unwanted ferromagnetic contamination from dust particles during the measuring procedure, a temporary clean-lab enviroment was made in the magnetometer area using sheets of polyethylene plastic. A remanent magnetization was induced in each sample prior to measurement by briefly exposing it to the field of a small CoSm magnet (maximum intensity 0·3 Tesla, T). Although these magnets have an inhomogeneous field, most of the volume of each sample was exposed to intensities in excess of 0·1 T, which is above the coercivity of all but the most highly elongate magnetite crystals. Tissue samples which displayed a measurable ferromagnetic remanence were then progressively demagnetized in an alternating magnetic field, using standard paleomagnetic techniques (see McElhinny, 1973, for detailed discussions of these methods). Ice-cube control samples made from glass-distilled water were magnetized and measured along with the experimental tissues as a control for laboratory generated contamination; no magnetic remanente was found in them. Estimates (± 10%) for the number of tumour cells in the experimental tissues were then made from histological sections, using a micrometer grid. In both the YC-8 lymphoma and Lewis lung tumour samples, between 5 and 10% of the volume was composed of blood vessels and connective tissue which was similar in appearance to that of the control samples.

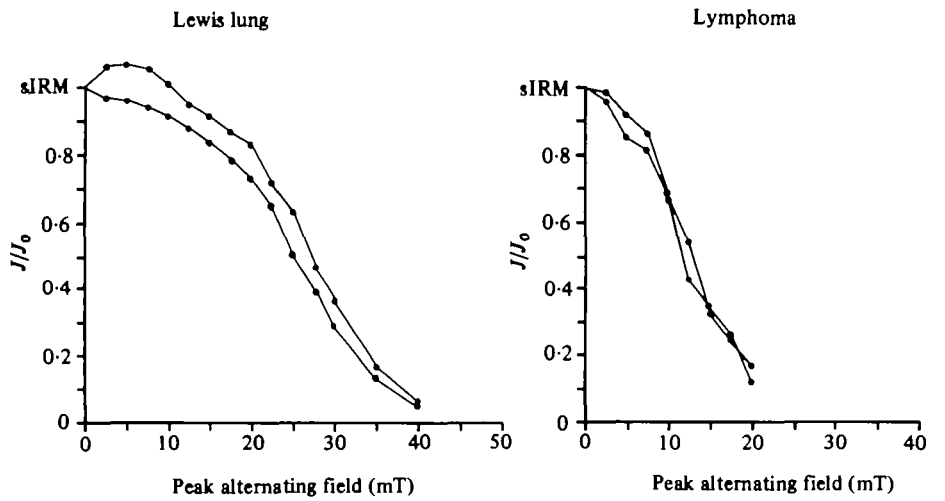

Fig. 1. Progressive alternating-field demagnetization of tumour tissues. Each frozen sample was initially exposed to the strong magnetic field of a CoSm magnet, producing a saturation Isothermal Remanent Magnetization (sIRM), which was then measured on the superconducting magnetometer. Each sample was then sequentially demagnetized along three perpendicular axes, starting with a 2·5 mT, 400 Hz field from a solenoid (Schonstedt Instrument Co.) and remeasured. This procedure was repeated, using progressively stronger peak alternating fields until most of the remanence had been destroyed. An assemblage of identical, non-interacting, single-domain grains treated in this fashion should produce a sigmoidal curve similar to those observed here. This implies that within each tumour type the particles are nearly identical, although YC-8 lymphoma and Lewis lung tumour form crystals of different size and shape.

Results from the magnetic analysis are displayed in Table 1 and Fig. 1. All samples of Lewis lung tumour and YC-8 lymphoma gave signals from 100 to 1000 times the background noise on the superconducting magnetometer, whereas samples from the normal mice and the human stomach, colon, and renal carcinomas were not notably ferromagnetic. Per cell, Lewis lung tumour is on the average nearly twice as magnetic as YC-8 lymphoma. Measurements on subdivided samples indicate that the magnetic material has a nearly uniform distribution. The progressive demagnetization experiments (Fig. 1) indicate that there are additional differences in the magnetic particles between the two cell types. Most of the ferromagnetic crystals in Lewis lung tumour have coercivities from 20 to 30 milli-Tesla (mT), whereas those in lymphoma cells are below 15 mT. Normally, the magnetic moment of such a particle is parallel to its long dimension, but it can point in either of two directions. For single-domain crystals, the coercivity is essentially the external magnetic field strength necessary to flip the direction of its remanent moment between these two positions. Although the coercivity of a ferromagnetic crystal is a complex function of its composition, size and shape, it generally increases with both particle elongation and volume. A comparison of these results with fig. 50 of McElhinny (1973) suggests that the Lewis lung tumour crystals are slightly larger or more elongate than those in YC-8 lymphoma cells, and that cells within one strain have nearly identical magnetic particles. Magnetite crystals made by a pure strain of magnetotactic bacteria are also remarkably regular in size

and shape (Balkwill, Maratea & Blakemore, 1980), although a great deal of variation exists between different types (Kirschvink & Gould, 1981). Similarly, the magnetic properties reported here are consistent with the interpretation that cells of each tumour type use the same genetic blueprint for constructing their magnetic crystals. This situation would be difficult or impossible to produce inorganically in nature.

Thermomagnetic, X-ray, Mössbauer, or electron diffraction analyses have identified magnetite as the source of the ferromagnetism in all organisms and tissues where it has been extracted, purified or otherwise examined to date (bacteria, Frankel *et al.* 1979; chitons, Lowenstam, 1962; honey bees, Gould *et al.* 1978; pigeons, Walcott *et al.* 1979; dolphins, Zoeger *et al.* 1981; tuna fish, Walker & Dizon, 1981; sea turtles, Perry, Bauer & Dizon, 1981). If magnetite is also the mineral responsible for the tumour remanence, the average volume present per cell would be $4{\cdot}5 \times 10^{-16}$ ml in Lewis lung tumour and $2{\cdot}5 \times 10^{-16}$ ml in YC-8 lymphoma, corresponding to cubes with dimensions of 77 and 61 nm respectively. Crystals of this size, if even slightly elongate in shape, will behave in general as single-domain ferromagnets, a property which is necessary for and consistent with the presence of a magnetic remanence in these tissues (Kirschvink & Gould, 1981). Although these data alone cannot determine the number of discrete crystals within each cell, individual particles less than about 35 nm in diameter would be superparamagnetic instead of single-domain and would not hold a stable magnetization. This puts an upper bound of about 11 and 6 crystals per Lewis lung tumour and YC-8 lymphoma cell, respectively.

Several problems arise, however, in the attempt to locate and identify these crystals using standard techniques on a transmission electron microscope (TEM). Firstly, there are only a few of these magnetic particles present per cell; consequently their relative density in the tissue is quite low. A typical 100 nm thick sample mounted on a TEM grid 1 mm in diameter, for example, would contain a total of only 10–70 crystals. Given their small size, these would be difficult to locate even though they are naturally electron-dense. The second problem is that other, much more abundant, subcellular components like pigment granules can have a similar TEM appearance. Small-area electron diffraction analysis (Towe & Moench, 1981) or X-ray fluorescence must be performed on each and every candidate particle to establish its crystalline nature and iron composition. Finally, any acidic step in the sample preparation would dissolve the small magnetite crystals. These factors probably explain why these particles have not been recognized in previous TEM studies. A more realistic initial approach might be to perform subcellular fractionation and centrifugation experiments and then use the magnetic remanence to pinpoint organelles associated with the ferromagnetic material. The tumour growth effects noted below together with the observations by Walsh, Shulman & Heidenreich (1961) of ferromagnetic inclusions in nucleic acid samples suggest that the nucleus would be a good place for an initial look.

At this stage, the biological function of these magnetic crystals also remains a mystery. The simplest hypothesis would be that they constitute some form of iron storage. Many tumours, however, have high internal concentrations of the iron storage protein ferritin (Dorner *et al.* 1980) and would not need to use magnetite for this purpose. Some forms have so much ferritin that radioisotope-labelled antiferritin antibodies have been used to locate tumours clinically (Order, Klein & Leichner,

81). It is interesting to note that the core of the ferritin macromolecule is made of the mineral ferrihydrite, the precursor of magnetite in chiton teeth (Kirschvink & Lowenstam, 1979). High ferritin levels might lead to magnetite precipitation as a by-product, but this theory does not explain the apparent crystal size uniformity nor the relatively small fraction of the cell's total iron content that is present in the ferromagnetic form. Ferritin is paramagnetic and cannot produce the observed remanence.

Many mouse tumours are known to be induced by viral action, and one possibility to consider is the production of magnetite under viral control. Although a virus has apparently not been found in association with Lewis lung tumour, YC-8 lymphoma is associated with a 45 nm RNA Moloney-sarcoma virus (MSV) in T-lymphocytes. This virus is probably too small to contain single-domain magnetite particles itself, but it might cause them to form somewhere else in the cytoplasm. In turn, this would indicate that the magnetic material is not in the nucleus.

Strong static or weak oscillating magnetic fields have been reported to influence adversely the growth rate of *in vivo* tumours (Batkin & Tabrah, 1977; Barnothy, 1964; Kim, 1976; Weber & Cerilli, 1971; Winterberg, 1967). Previously, these effects have been interpreted as arising from unspecified paramagnetic interactions or from induced ionic currents or ion motion in the cell cytoplasm. The presence of ferromagnetic crystals, however, provides at least two additional interpretations. Firstly, direct magnetomechanical motion of the crystals produced by their strong ($>>kT$) interactions with the applied magnetic fields and field gradients may damage adjacent subcellular organelles and, thereby, inhibit cell growth. Secondly, the relatively high, metallic-type electrical conductivity of magnetite, roughly 10^3 to 10^4 better than cytoplasm, may yield locally induced current densities that are strong enough to damage neighbouring structures, depending on the frequency and intensity of the applied electromagnetic fields.

Answers to these questions clearly await completion of the micro-anatomical and mineralogical characterization of the ferromagnetic particles. The discovery of the presence of ferromagnetic material in the YC-8 lymphoma and Lewis lung tumour cells led to further studies of the direct effects of magnetic fields on these tumour cells without the addition of paramagnetic compounds (in preparation).

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REFERENCES

BALKWILL, D. L., MARATEA, D. & BLAKEMORE, R. P. (1980). Ultrastructure of a magnetotactic spirillum. *J. Bact.* **141**, 1399–1408.

BARNOTHY, M. F. (ed.) (1964). *Biological Effects of Magnetic Fields*, vol. 1. New York: Plenum.

BATKIN, S. & TABRAH, F. L. (1977). Effects of alternating magnetic field on transplanted neuroblastoma. *Res. Comm. Chem. Pathol. Pharmacol.* **16**, 351–362.

BATKIN, S., TABRAH, F. L. & MISCONI, L. Y. (1980). The effect of Carbamoyltetramethylpyrilline-*N*-oxide on YC-8 Lymphoma cells *in vitro*. *Cancer Detection and Prevention* **3**, Abstr. 13.

DORNER, M. H., SILVERSTONE, A., NISHIYA, K., DE SOSTOA, A., MUNN, G. & DE SOSTOA, M. (1980). Ferritin synthesis by human T lymphocytes. *Science, N.Y.* **209**, 1019–1020.

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

GOREE, W. S. & FULLER, M. (1976). Magnetometers using RF-driven SQUIDS and their application in rock magnetism and paleomagnetism. *Rev. Geophys. Space Phys.* **14**, 591.

GOULD, J. L., KIRSCHVINK, J. L. & DEFFEYES, K. S. (1978). Bees have magnetic remanence. *Science, N.Y.* **201**, 1026–1028.

GROPP, C., HAVEMANN, K. & LEHMANN, F. G. (1978). Carcinoembryonic antigen and ferritin in patients with lung cancer before and during therapy. *Cancer* **42**, 2802–2808.

HANN, H. L., LEVY, H. M., EVANS, A. E. & DRYSDALE, J. W. (1979). Serum ferritin and neuroblastoma. *Proc. Soc. Clin. Oncol.* **20**, 126.

KEW, M. C., VORRANCE, J. D., DERMAN, D., SIMON, M., MACNAB, G. M., CHARLTON, R. W. & BOTHWELL, T. H. (1978). Serum tumor ferritins in primary liver cancer. *Gut* **19**, 294–299.

KIM, Y. S. (1976). Some possible effects of static magnetic fields on cancer. *T.I.T. J. Life Sci.* **6**, 11–28.

KIRSCHVINK, J. L. (1981). Ferromagnetic crystals (magnetite?) in human tissue. *J. exp. Biol.* **92**, 333–335.

KIRSCHVINK, J. L. & GOULD, J. L. (1981). Biogenic magnetite as a basis for magnetic field sensitivity in animals. *BioSystems* **13**, 181–210.

KIRSCHVINK, J. L. & LOWENSTAM, H. A. (1979). Mineralization and magnetization of chiton teeth: Paleomagnetic, sedimentologic and biologic implications of organic magnetite. *Earth Planet. Sci. Lett.* **44**, 193–204.

LOWENSTAM, H. A. (1962). Magnetite in denticle capping in recent chitons (*Polyplacophora*). *Geol. Soc. Am. Bull.* **73**, 435–438.

MCELHINNY, M. W. (1973). *Paleomagnetism and Plate Tectonics*. Cambridge University Press.

ORDER, S. E., KLEIN, J. L. & LEICHNER, P. K. (1981). Antiferritin IgG antibody for isotopic cancer therapy. *Oncology* **38**, 154–160.

PERRY, A., BAUER, G. B. & DIZON, A. E. (1981). Magnetite in the green turtle. *Trans. Am. geophys. Un.* **62**, 850.

PRESTI, D. & PETTIGREW, J. D. (1980). Ferromagnetic coupling to muscle receptors as a basis for geomagnetic sensitivity in animals. *Nature, Lond.* **285**, 99–101.

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

WALCOTT, C., GOULD, J. L. & KIRSCHVINK, J. L. (1979). Pigeons have magnets. *Science, N.Y.* **205**, 1027–1029.

WALKER, M. M. & DIZON, A. E. (1981). Identification of magnetite in tuna. *Trans. Am. geophys. Un.* **62**, 850.

WALSH, E. D., SHULMAN, R. G. & HEIDENREICH, R. D. (1961). Ferromagnetic inclusions in nucleic acid samples. *Nature, Lond.* **192**, 1041–1043.

WEBER, T. & CERILLI, G. J. (1971). Inhibition of tumour cell growth by the use of non-homogeneous magnetic fields. *Cancer* **28**, 340–343.

WINTERBERG, F. (1967). Some theoretical considerations on the inhibition of tumor growth by ultra-strong magnetic fields. *Arch. Biochem. Biophys.* **122**, 594–598.

ZOEGER, J., DUNN, J. R. & FULLER, M. (1981). Magnetic material in the head of the common Pacific Dolphin. *Science, N.Y.* **213**, 892–894.