Chapter 5

Detection, Extraction, and Characterization of Biogenic Magnetite

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1. Introduction

Several difficulties arise when attempts are made to characterize the deposits of magnetite found in metazoans. We are usually forced to deal with very small amounts of material, dispersed in tissues, using indirect methods that are subject to contamination. Magnetite crystals in the abdomens of bees (Gould *et al.*, 1978), and in the heads of pigeons (Walcott *et al.*, 1979), and other vertebrates (Bauer *et al.*, this volume; Perry *et al.*, this volume; Walker *et al.*, this volume) are submicroscopic (<100 nm), occupy a combined volume of 10^{-10} to 10^{-8} cm^3, and have a mass of 1–100 ng. In organisms of up to 100 kg or more, detecting such quantities of magnetite from its magnetic properties depends on the crystals being highly concentrated in small, recognizable structures, and not uniformly dispersed throughout all the tissues. Extraction and recovery of the crystals likewise depend on their being sufficiently concentrated to be magnetically detectable.

The failure to recognize contaminants and the influence they can have on results of biomagnetic studies has greatly hindered our progress in understanding the origin and functions of biogenic magnetite. Magnetite is a common industrial pollutant and can often find its way onto the external body surface or into the gut of higher animals (Kirschvink, 1983). A typical 100-nm crystal of the type found in bees and pigeons (Gould *et al.*, 1978; Walcott *et al.*, 1979) has a moment of about 0.5 fAm^2 whereas a 10-μm dust-sized particle

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may have moments of up to 500 pAm^2. The moment of the multidomain particle is well within the 1–10 pAm^2 sensitivity limits of the superconducting magnetometers currently in use whereas the moments of 10^3 to 10^4 of the single-domain particles must be aligned to be detectable. Other ferro- or ferrimagnetic contaminants are frequently present within the laboratory environment, particularly in paleomagnetic laboratories where rock samples tend to leave a fine dust that is often rich in magnetic contaminants. The ease with which contaminants can enter at all stages of biomagnetic studies dictates that we not only adopt procedures to minimize the risk of contamination but that we specifically distinguish between contaminants and true biochemical precipitates. It is therefore necessary to identify those properties that are likely to be unique to biogenic magnetite.

Interest in biogenic magnetite has focused on its potential use in the transduction of the geomagnetic field to the nervous system. Kirschvink and Walker (this volume) argue that the physical properties of the crystals are of primary importance and that single-domain crystals are the most likely form of magnetite for use in magnetoreception. This constraint should result in a restricted size-frequency distribution of the magnetite particles. Magnetite particles suitable for magnetoreception should therefore have coercivities greater than that of multidomain magnetite (<20 mT; Zoeger *et al.*, 1981) and less than the theoretical maximum for single-domain magnetite (300 mT; McElhinny, 1973). Superparamagnetic particles of biogenic magnetite (Gould *et al.*, 1978) are difficult to detect without special facilities. Thus, in searching for magnetite suitable for use in magnetoreception, we are primarily attempting to distinguish between single-domain and multidomain particles.

There is as yet no evidence that magnetite in the gut or the environment can naturally enter the bloodstream and be transported to the places it is detected. Any such particles used for magnetoreception are most likely produced within the organisms themselves, presumably by enzyme catalysis. The specificity of enzyme pathways would be expected to result in biogenic magnetite containing few of the impurities associated with geologic magnetite or metals used to harden iron alloys (Lowenstam and Weiner, 1983). Magnetite particles suitable for magnetoreception can therefore be reasonably expected to possess physical and chemical properties distinguishing them from geologic and synthetic magnetites.

Magnetite or magnetic material without apparent magnetoreceptive function has been detected in a variety of tissues in different species (Lowenstam, 1962; Presti and Pettigrew, 1980; Kirschvink, 1981; Kirschvink *et al.*, 1982). Except in the chitons, where magnetite is used to harden radular teeth (Lowenstam, 1962), the function of these deposits is unknown. Hypotheses are that the deposits store excess iron or that they may have a pathologic origin (Lowenstam and Weiner, 1983). It is therefore more difficult to predict characteristics that will distinguish them from other magnetites. However, it is important that attention be given to these deposits because they may predate the use of magnetite for magnetoreception (Kirschvink and Gould, 1981; Walker *et al.*, this volume).

This chapter reviews the techniques which are in common use for detecting and characterizing biogenic magnetite. Procedures for avoidance of contamination and examples of specific tests for contaminants that are easy to conduct are included. Finally, we attempt to identify further techniques that may prove useful in detecting, extracting, and analyzing biogenic magnetite.

2. Magnetometry Studies

As noted above and by other authors in this volume, accidental contamination of samples is a major problem in the search for biogenic magnetite. When working in paleo-

magnetic laboratories, we have sought to minimize the risks of contamination by thoroughly scrubbing and lining the walls, roof, and floor with thin polyethylene sheets. The recent development of a clean laboratory specifically designed for biomagnetic studies eliminates many of the contamination problems previously experienced (Kirschvink, 1983). We still find, however, that contamination can enter in lint on the clothing or in the hair of people using the laboratory. In the future, we hope to eliminate even this source of contamination by wearing lint-free garments and by using a deionized water shower.

Nonmagnetic tools are a must in carrying out dissections in preparation for measurements in the magnetometer. Typical metal dissection tools such as bone saws, scalpels, and forceps can leave trails of highly magnetic particles behind them. Even tools made from nonferrous metals such as aluminum or copper often contain small ferromagnetic inclusions sufficient to prevent their use in dissections. Magnetic particles left in tissues by these tools can easily be detected but can only be recognized from extensive tests of their magnetic properties (see below).

Wood, plastic, and glass seem to be the materials most suitable for tools used in dissections. We found glass microtome knives convenient for dissection and easily obtained from electron microscopy laboratories. Disposable wood chopsticks are ideal for handling tissue samples of the sizes measured in the magnetometer. Although they will acquire magnetic moments, frequent washing and replacement of the chopsticks minimizes the risk that they will pick up and transfer contaminants to samples. Frequent washing in glass-distilled water and extended ultrasonic cleaning in either glass-distilled water or 6 N HCl of nondisposable equipment that comes in contact with the samples also serve to minimize contamination.

The risk of contamination is further reduced if dissections are made from whole carcasses rather than sections that have been reduced in size using metal saws or knives (Walker et al., this volume). Saws appear to inject magnetic particles well into tissues. These particles may be dispersed further during dissection and their presence and contribution to tissue moments cannot be determined other than by extensive tests. We have also found from our work with fish that juvenile or subadult animals with incompletely ossified bones are easier to dissect than adults. In large, thick-boned species such as the blue marlin, *Makaira nigricans*, the easiest way to gain access to structures within the skull was to score along bone sutures with a glass knife and break the bones apart. In turtles and cetaceans, a rubber mallet or wooden-jawed vice was necessary to crack the skull bones to provide access to the dura mater membrane (Bauer *et al.*, this volume; Perry *et al.*, this volume). Such techniques, while effective, make accurate localization and identification of magnetite-containing structures difficult.

After tissue samples have been dissected and washed in glass-distilled water, several of their magnetic properties are of interest. These include the natural remanent magnetization (NRM), saturation isothermal remanent magnetization (sIRM), and rate at which magnetization is acquired or lost in progressively increased aligning or randomizing fields. Before a tissue sample can be measured, it must be frozen so that any small magnetic particles present will be immobilized. Otherwise, in the null field environment of the magnetometer, the orientation of any magnetic particles suspended in a viscous medium will be randomized by Brownian motion and any moments due to alignment of the particles will be lost.

Simply freezing a sample is sufficient only for measuring its NRM. Determination of other magnetic properties requires that the sample be magnetized prior to measurement. When inducing the sIRM in a sample, it is desirable to expose it to a strong uniform field in excess of 300 mT. In our early work we attempted to do this using a cobalt–samarium magnet. Unfortunately, it is difficult to obtain homogeneous magnetization of large samples using this method because of the rapid decay of field strength with distance from the magnet. Inhomogeneous magnetization can lead to underestimates of the amount of mag-

netic material present, making it possible to miss potentially important magnetic structures. An air core solenoid delivering homogeneous fields of up to several Tesla has proven very reliable in uniformly magnetizing samples for measurement in the magnetometer. This solenoid also makes possible the progressive magnetization of samples in coercivity studies (Kirschvink, 1983).

Great care is necessary in the choice of sample holders for magnetometry experiments. The mylar, glass, or polyethylene plastic tubes commonly used by paleomagnetists are adequate for use with biomagnetic samples, although they will acquire spurious moments and contribute to background noise in the magnetometer. They will also acquire magnetic moments if exposed to strong magnetic fields, so that samples must be magnetized separately from these holders and then loaded for measurement. Consequently, it is difficult to maintain the same orientation of the sample to components of the process (solenoids and magnetometer detection coils) in repeated measurements.

We have found two simple methods of attaching samples to a holder that maintain constant orientation of the sample relative to the axes of the solenoids and magnetometer pickup coils. A magnetized, frozen sample can be attached to the moistened end of a white cotton thread and lowered vertically into the magnetometer. If thoroughly cleaned, the thread does not show any NRM and can be used for repeated measurements such as alternating field (AF) demagnetization. However, the "clean" threads may sometimes gain moments if exposed to strong fields. Another method of loading the samples for rapid IRM acquisition studies was therefore required. We found that a more effective sample holder was a hook made of quartz glass fiber. The hook was inserted into the unfrozen tissue and left within it throughout all the measurements. With this apparatus and the air core impulse or AF solenoid mounted in line on the magnetometer, we were able to automate our experiments and minimize time spent handling samples during repeated measurements. Control experiments conducted with the quartz fiber hook attached to an ice cube showed that the fiber possessed no natural moment and did not acquire a moment even in high inducing fields. However, such fibers have the disadvantage that they are fragile and break easily.

There are several paleomagnetic techniques that can be adapted for identifying and characterizing the properties of biogenic magnetite. The most important of these is to determine the coercivity spectrum of the particles present in a tissue sample. The coercive field of a magnetite particle is the minimum intensity of an external field required to flip the moment of the particle from one of its stable orientations in the long axis of the particle to the other. The range of applied fields over which a sample acquires or loses magnetization is dependent on the coercivities of the magnetic particles present in the sample. Thus, the coercivity spectrum can eliminate a variety of minerals like hematite and goethite as possible sources of remanence, and can give information concerning the size and shape of any magnetite fraction present.

Two methods are available for determining the coercivity spectrum of particles present in a sample: progressive IRM acquisition and AF demagnetization. An external field, B, will shift the moments of magnetite particles with coercive fields less than $B \cos \theta$, where θ is the angle between the particle and field vector directions. In IRM acquisition experiments, the moments of particles in a sample are aligned by progressively increased fields. In AF demagnetization experiments, the orientation of aligned moments of particles with coercivities less than the peak applied field is randomized by sinusoidally oscillating fields which slowly decrease in magnitude. Although the use of the cotton thread or quartz fiber techniques described above makes these iterative measurements relatively easy, they make it impossible to correct for the effect of the angle between the direction of the crystal axes and the applied fields and cause slight overestimation of the coercivities of the particles. However, for distinguishing single-domain magnetite from multidomain particles, and also from high-coercivity minerals such as hematite, this error can be ignored. Thus, acquisition

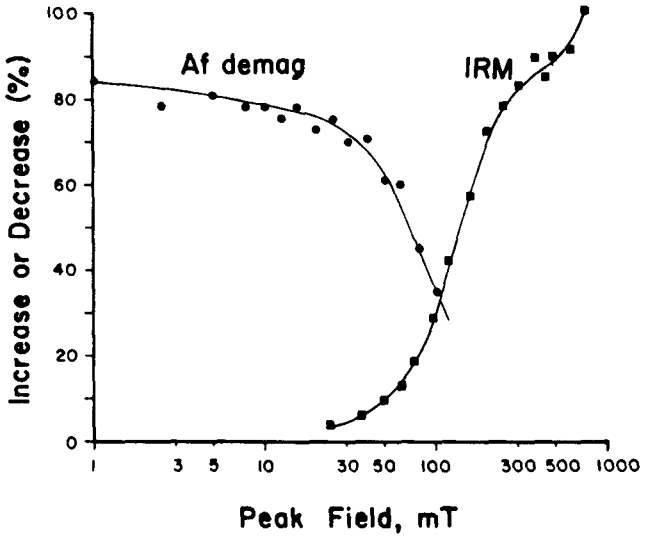

Figure 1. Comparison of IRM acquisition and AF demagnetization experiments on an ethmoid region sample from a human. 100% represents saturation magnetization and 0% represents the natural remanent magnetization of the sample. Sample supplied by R. R. Baker (Department of Zoology, University of Manchester).

of IRM in fields less than 20 mT or greater than 300 mT will indicate the presence of magnetic contaminants in samples.

Plotting the results of IRM acquisition and AF demagnetization experiments together can reveal much about the nature of the magnetic particles. For single-domain particles of the type predicted for use in magnetoreception, magnetization will be acquired or lost over a relatively small range of fields. Where all the particles are of single-domain size and uniformly dispersed throughout a sample, the two curves should be mirror images of each other over the same ranges of intensity of the applied fields. Where the crystals are sufficiently close to interact with each other, IRM acquisition is inhibited and AF demagnetization is aided (Cisowski, 1981). Thus asymmetry of the curves about the 50% magnetization point indicates the degree of interaction of the particles.

An example is given in Fig. 1 of the use of IRM acquisition to detect high-coercivity contaminants in a sample of bones and tissue from the ethmoid region of a human head. When we first tested this sample, we believed it had been dissected using nonmagnetic instruments. However, the sample turned out to be far more magnetic than any other biological sample we have examined (moments $>10^4$ pA m^2). The IRM acquisition curve was incompatible with the presence of magnetite alone, as the sample did not become saturated by 300 mT but continued to acquire magnetization up to fields of 800 mT. The AF demagnetization curve also showed the extraordinary stability of the magnetic material present in the sample. The tissue retained the majority of its magnetization up to fields of 100 mT, the upper limit of our demagnetization unit. From these data we could only conclude that there must have been high-coercivity contaminants in the tissue. On checking, R. R. Baker (Department of Zoology, University of Manchester, personal communication, 1982) discovered that an assistant had inadvertently trimmed the sample with a band saw. It would thus be impossible to conclude that biogenic magnetic particles contained within the sample had been detected and identified as magnetite suitable for use in magnetoreception.

Where suitable facilities are available, several other techniques have proven or should prove useful in identifying and characterizing magnetite present in biologic samples. The identity of the mineral can be determined from its Curie temperature, a procedure used to identify magnetite in pigeons and honeybees (Gould *et al.*, 1978; Walcott *et al.*, 1979).

The loss of IRM on warming through the isotropic point of magnetite has been used to identify multidomain magnetite in the Pacific dolphin (Zoeger *et al.*, 1981). As it distinguishes single-domain from multidomain material, this test is potentially very useful in demonstrating the presence of contaminants in biologic samples. Finally, the presence of superparamagnetic crystals can be demonstrated by continuously monitoring the remanent moment of a sample versus temperature as it warms from liquid nitrogen temperature (77°K) to room temperature (293–298°K). Freezing the samples to liquid nitrogen temperature shifts the boundary between single-domain and superparamagnetic behavior toward smaller particle sizes. Thus, as the crystals warm through their single-domain/superparamagnetic transition, they will lose their stable remanence. A drop in total moment will accompany this transition and the temperature at which it occurs will indicate the approximate sizes of the crystals. This experiment demonstrated the presence of over 10^8 such particles with sizes of 30–35 nm in the honeybee (Kirschvink and Gould, 1981) as well as in one species of chiton (Kirschvink and Lowenstam, 1979).

3. Extraction and Characterization of Biogenic Magnetite

Much can be learned about the nature and organization of the magnetic material discovered in biologic samples using its bulk magnetic properties as discussed above. However, it is eventually necessary to extract the material from the sample and apply a range of techniques to its identification and characterization. Areas of highest magnetite concentration must be accurately identified if sufficient quantities of material are to be obtained for analysis. In the fish this was done by magnetometric studies that exhaustively sampled the tissues of one species, the yellowfin tuna, *Thunnus albacares*, until tissues containing high magnetic remanence could be reliably located (Walker and Dizon, 1981; Walker *et al.*, this volume). We were able to identify one specific and relatively small structure, the dermethmoid bone, that was always magnetic in the tuna and in all the fish species subsequently examined. Our experiments showed that the magnetite was concentrated in tissue contained within a sinus formed within the dermethmoid bone.

In other vertebrates, magnetite appeared to be concentrated in the dura mater (Bauer *et al.*, this volume; Perry *et al.*, this volume) or in a region of the skull similar to the fish (Mather and Baker, 1981; Baker *et al.*, 1983; Baker, Chapter 26, this volume; Mather, this volume). In the turtles and cetaceans, subdivision of the dura revealed localization of magnetic material within its anterior portions. Concentration on these regions made for efficient extraction of magnetite in the green turtle, *Chelonia mydas* (Perry *et al.*, this volume).

For invertebrates the situation is less clear. Deposits of magnetite or magnetic material have been located from magnetometry and coercivity studies in the abdomen of the honeybee (Gould *et al.*, 1978) and in the head-thorax of the monarch butterfly (Jones and MacFadden, 1982). There appears to be no reason why magnetite should not be detected in the bodies of other invertebrate groups. If easily accessible magnetite-containing structures can be identified, the generally smaller size of invertebrates compared to vertebrates could make them more suitable for magnetite extraction studies.

Once the magnetic structures have been identified, it is a simple matter to dissect and combine a number of them for magnetite extraction. In this way we were able to treat the dermethmoid tissues of up to five yellowfin tuna at once. The tissues were ground with a little distilled water in a glass tissue grinder or in a test tube using a nonmagnetic pestle. In the tuna this released fat and oil droplets into suspension. These were removed by adding anhydrous ether to the suspension and shaking vigorously. After the aqueous and ether phases separated, the ether was decanted and replaced. This procedure was repeated until the aqueous phase became clear.

After ether extraction, the suspension was centrifuged, the aqueous supernatant removed, and 5% Millipore* filtered hypochlorite solution (commercial bleach) added. The mixture was centrifuged and the hypochlorite replaced periodically either until the tissue disappeared completely or until no further digestion occurred. When digestion was complete, the suspension was centrifuged and the supernatant replaced with distilled water. This washing procedure was repeated at least five times. In the tuna a white residue associated with the magnetic material remained after tissue digestion. Treatment with buffered EDTA (pH 7.1) carried out in similar fashion to the hypochlorite digestion freed the crystals from the residue. They could then be separated magnetically from the residue under a dissecting microscope.

To remove the magnetic particles for analysis, we held them in suspension using a cobalt–samarium magnet held to the side of the test tube, and then pipetted them onto a slide coated with xylene-based cement. The water in which the crystals were pipetted onto the slide was allowed to evaporate and a second layer of cement covering the first applied. The crystals were thus sealed in a cement sandwich which could be cut out, removed from the slide, and placed in the beam of a mini-Debye Scherrer X-ray camera. For electron microprobe analysis we pipetted the crystals onto clean microscope slides, allowed them to dry completely, and transferred them to slides coated with epoxy resin. It was then a simple matter to cure the resin, polish the crystals, and coat them with carbon for electron microprobe analyses. Similarly, aggregates can be pipetted onto plugs and prepared for examination in the scanning electron microscope (SEM) (see Perry *et al.*, this volume).

We exposed the crystals to Mo K_α X-irradiation (48–72 hr) in the mini-Debye Scherrer camera. Development of the film and measurement of the band patterns was then routine (see Perry *et al.*, this volume; Walker *et al.*, this volume). The procedure for electron microprobe analyses was more complex. These analyses determine the elemental composition of minerals and permit tests of the purity and origin of the magnetite crystals. A survey using EDAX probe was an appropriate beginning point as it indicated all the elements present and their relative proportions in any sample (see Perry *et al.*, this volume). Key elements were then selected for quantitative analyses. In the tuna and turtle, we analyzed for oxides of iron against a magnetite standard and for oxides of rare earth metals such as titanium and manganese, which are commonly found as impurities in geologic magnetite (Perry *et al.*, this volume; Walker *et al.*, this volume). We also chose to analyze for calcium in an attempt to determine how closely the residue remaining after hypochlorite digestion was associated with particle aggregates in the tuna.

Our analyses of the magnetite from the green turtle and the tuna showed that there were very few oxides other than oxides of iron in the material. Although the standard (NMNH 11487) used in the quantitative analyses was an unusually pure geologic magnetite (M. O. Garcia, Hawaii Institute of Geophysics, University of Hawaii, personal communication), it still contained measurable oxides of the rare earth metals titanium and chromium, whereas the biologic magnetites did not (see Perry *et al.*, this volume; Walker *et al.*, this volume). These data, taken with the absence of nickel from the turtle samples (Perry *et al.*, this volume) and very small amounts of manganese in magnetite from both the tuna and the turtles, strongly suggested to us that the magnetite was neither synthetic nor geologic in origin.

The very small amounts of material obtained by digestion made it impossible to use the approach developed by Towe (this volume) for preparing the crystals for transmission electron microscopy (TEM). Chang (Division of Geology and Planetary Science, California Institute of Technology, personal communication) developed a method for obtaining dis-

* Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

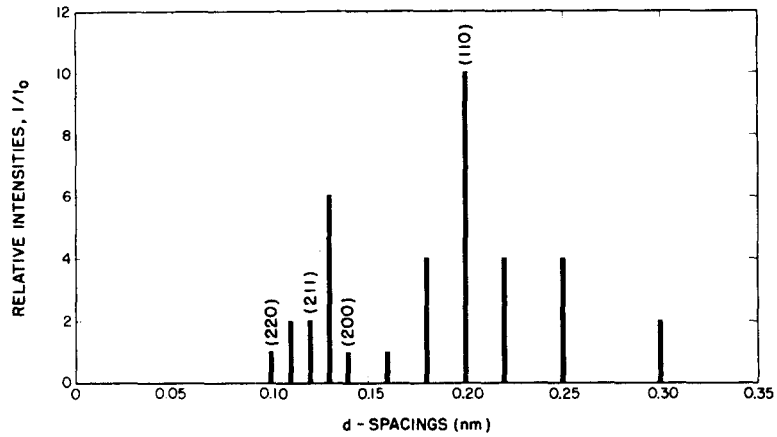

Figure 2. X-ray diffraction data for magnetic particles extracted from mouse tumors supplied by F. L. Tabrah and S. Batkin (John A. Burns School of Medicine, University of Hawaii). Vertical lines indicate relative intensities of the lines in the diffraction pattern. Numbers in parentheses indicate lines associated with metallic iron and the crystal plane giving rise to each line. Lines at *d* spacings of 0.30 and 0.25 nm could come from either magnetite or maghemite. The sources of the remaining lines in the pattern are unknown.

persed crystals that compensated for this limitation. The aggregated crystals were pipetted onto carbon-coated copper mesh grids and dispersed in an alternating magnetic field (100 mT). The grids were then air-dried and prepared for examination in TEM (see Walker *et al.*, this volume).

Extraction of the magnetic material immediately produced a substantial amount of information that assisted in its identification. For example, the color of the particles extracted from the tuna and the turtle was sufficient to exclude maghemite. Maghemite has magnetic properties similar to magnetite and therefore cannot be excluded as a source of magnetic remanence by coercivity studies. However, tests were still necessary to identify the crystals uniquely, demonstrate their biologic origin, and to exclude the possibility that contaminants may have entered during dissection or extraction. For example, the particles extracted from the yellowfin tuna and green turtle were uniquely identified as magnetite by X-ray diffraction (Perry *et al.*, this volume; Walker *et al.*, this volume). In contrast, X-ray diffraction of magnetic material extracted from tumors that had been shown to contain probable single-domain magnetite crystals revealed native iron, and a mineral that could have been magnetite or maghemite (Fig. 2). On the basis of color, maghemite could be excluded. However, the iron was presumably a contaminant arising from an as yet unrecognized source.

Diffraction patterns are not conclusive proof of the origin of magnetite particles extracted from tissues, and care is necessary in their interpretation. Pure, fine-grained magnetite powders such as those predicted for use in magnetoreception will ideally yield sharp, unambiguous diffraction patterns. Streaking of the spots or lines in X-ray and electron diffraction patterns could arise from more than one source. Towe and Moench (1981) suggest that vacancy defects in the crystal structure could have caused streaking of an electron diffraction pattern taken from single-domain magnetite crystals purified from magnetotactic bacteria. However, multidomain particles could also give streaked diffraction patterns. Therefore, procedures that combine identification of the particles with determination of their domain state are necessary. Electron diffraction and measurement of the

size and shape of isolated crystals can be conducted on the same samples mounted on copper mesh grids. Thus, although electron diffraction is a more cumbersome technique than X-ray diffraction, it does provide a conclusive test of the origin of the particles when carried out in conjunction with determination of their size and morphology (Towe, this volume; Walker *et al.*, this volume).

4. Discussion

The magnetometric methods developed so far permit detection and characterization of the bulk properties of concentrations of magnetite in organisms. It is also now possible to conduct analyses of polycrystalline aggregates and isolated crystals of magnetite extracted from magnetic tissues. An important conclusion arising from these studies is that although it is relatively easy to detect the presence of magnetic material in organisms, it is far more difficult to determine its origin and what, if anything, it does. We have attempted to define a theoretical basis for determining what the form of biogenic magnetite used in magnetoreception should be, and the experimental evidence to date has been consistent with the theory. This permitted refinement of the hypothesis on the organization of magnetite-based magnetoreceptor organelles (Kirschvink and Gould, 1981; Kirschvink *et al.*, in press; Kirschvink and Walker, this volume; Walker *et al.*, this volume), providing opportunities for further tests of the ferromagnetic magnetoreception hypothesis.

An important result of this work is the demonstration of the need to test for contaminants at all stages of the research, and to check for consistency of results obtained from different techniques. The differences between the bulk magnetic properties of the dura mater of the Pacific dolphin (Zoeger et al., 1981), the sample shown in Fig. 1, and the ethmoid tissues of pelagic fish (Walker *et al.*, this volume) illustrate quite clearly the effects contaminants can have on magnetometry results. The mouse tumors from which we extracted magnetic particles had previously been shown to be magnetic and probably to contain single-domain magnetite (Kirschvink *et al.*, 1982). X-ray diffraction of the magnetic particles extracted from one of the tumor strains demonstrated the presence of native iron, with the possible presence of magnetite. The presence of iron conflicts with the magnetometry data and suggests that contaminants may have entered during the extraction. Thus, magnetic material detected in tissues and obtained by extractions should be shown to be biogenic by independent means wherever possible.

The property of biogenic magnetite crystals that truly sets them apart from their synthetic and geologic counterparts is their nonoctahedral crystal morphology in TEM (Towe and Moench, 1981; Matsuda *et al.*, 1983; Walker *et al.*, this volume), a property not essential to the magnetite-based magnetoreception hypothesis. This observation suggests that the distinctive properties of biogenic magnetite arise from the biomineralization process. In line with this, it seems reasonable that the chemical composition of biogenic magnetite would be different from geologic magnetite. A potentially much more important characteristic is the ratio of the oxygen isotopes present in biologic compared with nonbiologic magnetite (Lowenstam and Kirschvink, this volume). At present, technology and availability of material limit the utility of oxygen isotopes for the recognition of biogenic magnetites. Currently available mass spectrometers require a minimum of about 20 μg of sample, which is the equivalent of quantitative extraction of the magnetite from the dermethmoid tissue of at least 100 yellowfin tuna. The potential for entry of contaminants in such a large-scale extraction is enormous. An ion microprobe may require less sample than a mass spectrometer and we are investigating the adaptation of this apparatus for analysis of biogenic magnetites.

Thus, a substantial case can be made for our ability to determine what is and is not biogenic magnetite suitable for use in magnetoreception. This does not imply that the case

for magnetite-based magnetoreception is proven. However, the approach that has helped in recognition of magnetite suitable for magnetoreception may prove useful in predicting the important properties of other deposits of magnetite found in metazoans. Characterization of these deposits is important because they may predate the use of magnetite in magnetoreception (Kirschvink and Gould, 1981; Walker *et al.*, this volume), and because it is necessary to be able to distinguish the two from each other and from contaminants.

Simple hypotheses for the origin of biogenic magnetite are that it constitutes some form of iron storage or is deposited pathologically (Lowenstam and Weiner, 1983; Walker *et al.*, this volume). In support of these hypotheses is the observation that anomalous deposits of magnetic material are found more frequently in larger and older organisms (J. G. Mather, Department of Zoology, University of Manchester, personal communication, 1981; Perry, unpublished data; Walker, unpublished data). Crystal size and form may be less important in these cases than in magnetite used in magnetoreception. Presumably, enzyme catalysis will be necessary to precipitate the mineral, so the deposits are still likely to be chemically distinctive. Study of the biomineralization of other storage or pathologically formed minerals may be instructive in helping predict the likely properties of biogenic magnetite not used in magnetoreception. This may also assist in predicting where such deposits are likely to form and in their subsequent detection.

By carrying out studies with magnetite specifically in mind, we risk failing to recognize other interesting biologically formed magnetic minerals. In recent years the number of known biogenic minerals has increased substantially (Lowenstam, 1981; Lowenstam and Kirschvink, this volume). In carrying out biomagnetic studies, we should therefore be prepared to adopt appropriate approaches for correct identification and characterization of other magnetic minerals. The basic magnetometric techniques suitable for detecting these minerals are already well established through paleomagnetic studies. Buffered enzyme extractions aimed at digesting specific components of magnetic tissues may be a suitable approach for extracting magnetic minerals unaltered. The specific methods for detecting, extracting, and characterizing biogenic magnetite have undergone considerable development in the last year or two. We expect this development to accelerate as interest in biomineralization grows and hope that this chapter serves as a stimulus to development of new, more efficient approaches.

5. Summary

Magnetite detected in cells or tissues is part of a recently described class of biologically formed minerals and provides a possible physical basis for magnetoreception in living organisms. Detection of magnetic material alone adds little to understanding of either the biomineralization process or the possible use of the material in magnetoreception. Further progress requires development of procedures that will distinguish between magnetic contaminants and biologic precipitates in tissue. Hypotheses concerning the nature and role of the magnetic material can then be tested.

This chapter considers the special case of magnetite that is likely to be used in magnetoreception and attempts to define properties that will distinguish it from geologic and synthetic magnetites. Methods developed to detect and characterize the magnetic properties of such material and to distinguish it from contaminants are then described. A similar approach is used to develop techniques for extraction, identification, and characterization of the properties of individual crystals and crystal aggregates. Examples of tests that have identified magnetic material other than magnetite that could be used in magnetoreception are given for magnetometry, extraction, and histologic studies.

The studies demonstrate that the biomineralization process is responsible for producing the key distinguishing features of biologically formed magnetite. Although invasive

and noninvasive techniques can distinguish between biologic and nonbiologic magnetite, use of both approaches at once can provide considerably more information on the nature of the magnetic material as well as checks on results obtained by either set of techniques. Extension of the approaches developed in this chapter to other magnetic minerals with other possible roles in the physiology of the living organism could be equally fruitful.

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