A Candidate Magnetic Sense Organ in the Yellowfin Tuna, *Thunnus albacares*

Abstract. *Single-domain magnetite crystals have been isolated and characterized from tissue located in a sinus within the dermethmoid bone of the skull of the yellowfin tuna,* Thunnus albacares. *Their chemical composition, narrow size distribution, and distinctive crystal morphology indicate that these crystals are biochemical precipitates. Experiments on the interaction between particles reveal the organization of the particles in situ and suggest a possible form for candidate magnetoreceptor organelles. The consistent localization of such particles with similar arrangement within the dermethmoids of this and other pelagic fishes suggests that the ethmoid region is a possible location for a vertebrate magnetic sense organ.*

Magnetic material has been detected in the tissues of various metazoan species (*1–5*). Although the material is inferred to be magnetite, in many cases this has not yet been established, and external contaminants have not been excluded as possible sources of magnetic remanence. Even in the homing pigeon and the honey bee, detailed localization of the magnetite has proved difficult to ascertain, and the particles have not been isolated or characterized previously (*3, 4*). For many of the species studied, behavioral evidence for magnetic sensitivity is lacking or in dispute.

Earlier we reported reproducible conditioned responses to earth-strength magnetic fields in the yellowfin tuna, *Thunnus albacares* (*6*). We now report the detection, extraction, and characterization of magnetite crystals from tissue within a sinus formed by the dermethmoid bone of the skull of this species. The crystals have a narrow size distribution, are single magnetic domains, and have morphologies similar to other biochemically formed magnetites. Studies of the interactions between particles suggest that the crystals are arranged in groups or chains in the dermethmoid tissue. Magnetite-based magnetoreceptor organelles arranged in vivo in a form consistent with these observations could provide these fish with a sensitive magnetoreception system.

To distinguish magnetic material with a possible magnetoreceptive function from other deposits, we sought to identify a tissue with the following characteristics: (i) it should have a high remanent magnetic moment concentrated in a small volume of sample compared with other tissues from the same fish; (ii) the anatomical position of the magnetic tissue must be consistent from fish to fish; (iii) the bulk magnetic properties, including particle coercivity, should be similar in different individuals and in different species of fish; and (iv) it should be innervated.

Tissue and organ samples, including bones of the body and the skull, skin, sense organs, viscera, and swimming muscles, were dissected from three 1-year-old yellowfin tuna (fork length, 40 to 50 cm) with glass microtome knives and handled with nonmetallic tools in a magnetically shielded, dust-free clean room. Although subsequent dissections focused on the most magnetic tissue, other samples were measured in all fish. Samples were washed in glass-distilled water, frozen in liquid nitrogen, exposed to strong fields from a cobalt-samarium magnet or an air-core impulse solenoid (*7*), and tested for isothermal remanent magnetization (IRM) in a superconducting magnetometer. We extracted the magnetic material for other tests by combining the magnetic tissue from several fish, grinding the tissues in a glass tissue grinder, extracting released fats with ether, digesting the remaining cellular material in Millipore filtered 5 percent sodium hypochlorite solution (commercial bleach), and briefly treating the residue with 0.5*M* EDTA (*p*H 7.1). After centrifuging and washing, aggregates of black particles could be separated magnetically from the residue; control samples of originally nonmagnetic tissues yielded no such product. The magnetic

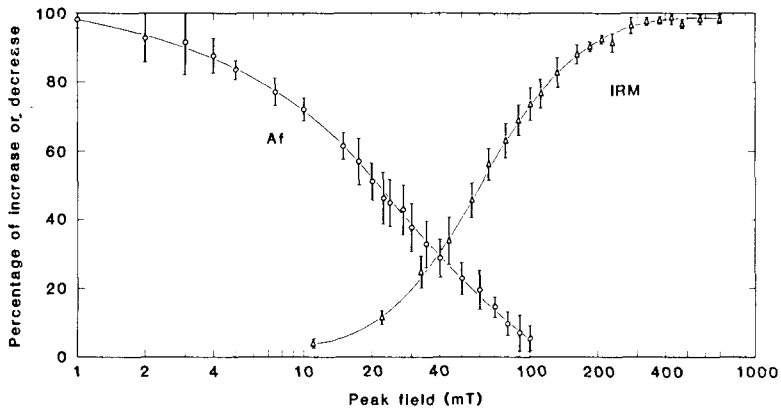

Fig. 1. The one-axis Af demagnetization and IRM acquisition curves for the dermethmoids of four yellowfin tuna. Saturation magnetization (1 to 3 × 10^3 pA·m^2) is 100 percent and natural remanent magnetization is 0 percent in both plots. Error bars represent standard errors of the means.

powder extracted from the dermethmoid tissue was analyzed by x-ray diffraction, electron microprobe, and transmission electron microscopy (TEM).

Of 17 tissues and organ samples examined for magnetic remanence, 15 had mean moments less than 500 pA·m^2, and two (eye tissue and dermethmoid bone) had moments greater than 1000 pA·m^2 (*8*). The intensities of magnetization of these samples identified the frontal and dermethmoid bones as the samples containing the greatest concentrations of magnetic material (*8*). Subdivision and remeasurement of the dermethmoids from a number of fish suggested that the magnetic material was contained in a sinus formed within the dermethmoid bone. Because the dermethmoid bones acquired greater moments (260 to 3000 pA·m^2) than the frontal bones (59 to 300 pA·m^2) and were always clearly magnetic, we focused our remaining studies on the dermethmoid bone and on the tissue it contained in particular.

The frozen dermethmoid tissues of seven yellowfin tuna had natural remanent magnetization moments at or below the instrument noise level (< 50 pA·m^2). We magnetized these samples (600 to 3000 pA·m^2) and allowed them to warm to room temperature, measuring their moments at 5-minute intervals. The moments retained by the samples all decayed with time, although not all lost their moments completely within the period of the experiments (1 hour). This observation suggests that, as the tissues thawed, the orientation of the magnetic particles became randomized through thermal agitation.

We washed and refroze the dermethmoids of four fish, subjected them to magnetic fields of progressively increasing strength with the impulse solenoid, and then demagnetized them with progressively increasing alternating fields (Af). The magnetic moment remaining after each step in these procedures was measured in the magnetometer. The dermethmoids acquired virtually all of their magnetization in fields between 10 and 200 mT and lost it again in alternating fields between 10 and 100 mT (Fig. 1). The absence of the multi-domain magnetite particles detected by Zoeger *et al.* (*5*) in the Pacific dolphin, *Delphinus delphis*, is indicated by the flatness of the Af demagnetization curve below peak fields of 10 mT. The almost complete saturation of the samples in fields above 200 mT rules out the presence of hematite and metallic iron alloys, which will continue to acquire remanence in fields above 1000 mT.

If the magnetic particles producing the moment were uniformly dispersed throughout the dermethmoid tissue, the IRM acquisition and Af demagnetization curves would be symmetrical about the 50 percent magnetization point. This follows because magnetic moments that are aligned by a given impulse field level should also be moved by an alternating field of the same strength. Interactions between the particles aid Af demagnetization and inhibit IRM acquisition, displacing the curves and causing their intersection to fall below the 50 percent magnetization point (*9*). However, the abscissa of the intersection point still provides a good estimate of the median coercivity of magnetic particles in the sample (*9*). The ordinate and the abscissa of the intersection of the Af demagnetization and IRM acquisition curves for the yellowfin tuna dermethmoids were at 30 percent magnetization and 40 mT, respectively (Fig. 1). These data are compatible with the presence of about 8.5 × 10^7 single-domain magnetite particles in the dermethmoid tissue; these particles are approximately 50 nm in length, have axial ratios of about 0.8 (*10*), and are organized into interacting groups or chains (*9*, *11*).

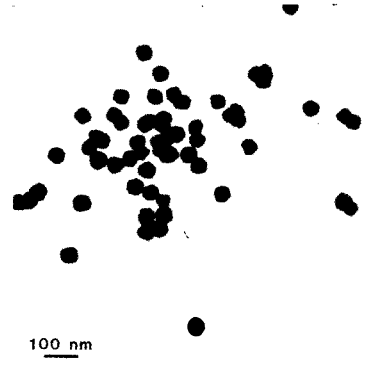

Fig. 2. Isolated magnetite crystals from the yellowfin tuna dermethmoid tissue. Scale bar is 100 nm.

An x-ray diffraction pattern identified magnetic particles extracted from the dermethmoid tissue as crystalline magnetite (*12*). Electron microprobe (Cameca MBX) analysis showed that the crystals were pure, containing no measurable titanium, chromium, or manganese (*11*). In TEM, the isolated crystals were 45 ± 5 nm in length and 38 ± 5 nm in diameter (mean ± standard error of the mean) (Fig. 2). These dimensions fall within the single-domain stability field of magnetite (*13*), and their sizes and axial ratios match the particle coercivities measured in whole tissues. The crystals do not conform to the octahedral crystal morphology or lognormal size-frequency distributions normally shown by geologic or synthetic magnetites (*14*). Nonoctahedral crystal habits and uniform size distributions are characteristic of chiton and bacterial magnetites (*15*), which suggests that crystal morphology is a useful means of distinguishing biologic from nonbiologic magnetites (*15*, *16*).

The properties and organization of the magnetite particles in the dermethmoid tissue of the yellowfin tuna meet preconditions for use in magnetoreception and suggest a possible form for magnetite based magnetoreceptors. Their chemical composition, uniform size, and biologically distinctive morphology are evidence of closely controlled biomineralization processes and, consequently, magnetic properties. The crystals will have a coupling energy with the geomagnetic field of about 0.1 kT. They are therefore too small to contribute individually to magnetoreception since their net

alignment, as given by the Langevin function, will be poor (*17*). Organization of the particles into chains similar to those in the magnetosomes of magnetotactic bacteria (*18*) will yield greater coupling energies and is consistent with the interactions between the particles detected in the dermethmoid tissue. The decay with warming of the IRM acquired by the dermethmoid tissue indicates that the particle groups are at least partially free to rotate. Taken together, these results suggest an association of the particles with a mechanoreceptor that detects the position or movement of the groups. Theoretical analyses (*19*) show that chains of 20 to 60 particles would provide ideal coupling energies with the geomagnetic field for use in magnetoreception. Assuming that the 8.5×10^7 particles detected in the dermethmoid tissue are arranged in such a fashion, a magnetite-based magnetoreception system in the yellowfin tuna could resolve magnetic field direction to within a few seconds of arc, or magnetic field intensity differences of 1 to 100 nT (*19*).

Gross dissection of the dermethmoid region of the yellowfin tuna revealed the supraophthalmic trunk nerve, which carries branches of the trigeminal, facial, and anterior lateral line nerves and which ramifies in the ethmoid region. Histological studies have suggested the presence of nerve axons in the dermethmoid tissue (*20*). A suitable physical and possible neural basis for previously demonstrated behavioral responses to magnetic fields has thus been demonstrated for the first time in one species. Our magnetometry results are consistent in phylogenetically distant fishes (*12*) and, along with similar results for other vertebrates (*1*, *4*, *5*), suggest that the ethmoid region of the skull is a likely site for a vertebrate magnetic sense organ.

Note added in proof: Magnetite crystals isolated from the dermethmoid tissue of chinook salmon, *Oncorhynchus tshawytscha*, are organized in chains when viewed in TEM (*21*).

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