Chapter 20

Magnetoreception and Biomineralization of Magnetite Fish

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1. Introduction

Many species from different taxa respond to one or more features of the geomagnetic field (Keeton, 1971, 1972; Lindauer and Martin, 1972; Wiltschko, 1972; Walcott and Green, 1974; Martin and Lindauer, 1977; Quinn, 1980; Wiltschko *et al.*, 1981). These responses fall into two general categories: responses to magnetic field direction and to magnetic field intensity. Magnetic compass responses include the vanishing bearings of homing pigeons (Walcott and Green, 1974) and directional preferences of migratory species in orientation arena experiments (Wiltschko, 1972; Tesch, 1974; Quinn, 1980). The postulated magnetic intensity, or "map", response (Gould, 1980, 1982; Moore, 1980; Walcott, 1980) refers to the apparent ability of homing pigeons to determine their position to within a kilometer or two using some feature related to geomagnetic field intensity. This response has been inferred from the vanishing bearings and homing speeds of birds released at geomagnetic field anomalies and during magnetic storms (Keeton, 1969, 1971, 1972; Gould, 1980, 1982; Walcott, 1980). Gould (Chapter 12, this volume) provides a full discussion of this research.

Experimental evidence is accumulating that fish also possess a magnetic compass and that they can learn to respond to magnetic fields in conditioning experiments. Quinn

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(1980), Quinn *et al.* (1981), and Quinn and Brannon (1982) demonstrated unconditioned compass responses in sockeye salmon fry and smolts. Fish held in the center of four-armed wooden tanks (Quinn, 1980; Quinn *et al.*, 1981), or circular arenas with eight escape traps (Quinn and Brannon, 1982) were released and allowed to enter the tank arm or escape trap corresponding to their directional preference. In the absence of visual cues, the fish made their preferences using magnetic field cues. These preferences were appropriate for bringing the fish to their nursery lake or its downstream outlet.

Kalmijn (1978) conditioned a response to magnetic field direction in the ray, *Urolophus halleri*, in which the fish were rewarded for entering an enclosure in the magnetic east and punished for entering an identical enclosure placed in the magnetic west of the experimental tank. When the magnetic field direction in the tank was reversed randomly, the fish selected the enclosure in the magnetic east of the tank with accuracies of up to 90% in training sessions.

There are two central problems in the study of magnetic sensitivity in animals. The first is that many of the behavioral results obtained so far are subject to methodological criticisms, may be unreproducible, and tell little about the functioning of the sense (Emlen, 1975; Able, 1980; Griffin, 1982). The second is that as yet it is unknown how and where the magnetic field is detected (Able, 1980). Thus, it is difficult to design and conduct explicit experiments to obtain the necessary behavioral, anatomical, and neurophysiological proofs of the existence of the sense, and to analyze its capacities.

Conditioning experiments can provide the necessary reproducibility and power for unequivocal demonstration of the existence of a magnetic sense. However, attempts to condition animals to magnetic fields have largely failed (Able, 1980). Where conditioning has been obtained (Reille, 1968; Bookman, 1977; Phillips, 1977; Kalmijn, 1978), either the experiments were unreproducible (Kreithen and Keeton, 1974; Beaugrand, 1976; Griffin, 1982) or subsequent psychophysical analyses of the sense were not carried out (Phillips, 1977; Kalmijn, 1978). These inconsistent results suggest that the experimental designs may have been inappropriate for demonstrating responses to magnetic fields (Ossenkopp and Barbeito, 1978). Thus, it is not yet possible to reject or accept without reservation the hypothesis that animals can detect magnetic fields. Further attempts must be made to obtain robust, reproducible responses to magnetic field stimuli and to identify their sensory bases.

Any hypothesis seeking to explain geomagnetic field sensitivity in animals must provide a mechanism by which the action of the geomagnetic field can bring about orderly displacement of the electrical potential of a receptor cell membrane. That is, the geomagnetic field must act on the magnetoreceptor cell with a neural coupling energy greater than the background thermal energy, kT (Jungerman and Rosenblum, 1980). The mechanism must also explain both the general compass response and the very high sensitivities inferred for detection of changes in magnetic field intensity (Martin and Lindauer, 1977; Gould, 1980, 1982). Finally, the hypothesis should make testable predictions concerning magnetoreceptor operation.

Among the hypotheses for magnetoreception that have been suggested are forms of electrical induction (Kalmijn, 1974; Jungerman and Rosenblum, 1980); optical pumping (Leask, 1977); liquid crystal effects (Russo and Caldwell, 1971); and biological superconductivity (Cope, 1971, 1973). These hypotheses demonstrate stimulus energies sufficient to depolarize the membrane of a receptor cell and make geomagnetic field detection possible. However, few explain both the directional responses to magnetic fields made by animals and the sensitivity to very small variations in magnetic field intensity exhibited by homing pigeons and other birds (Southern, 1978, Gould, 1980, 1982; Walcott, 1980). In addition, there may be a lack of evidence for receptor cells which behave in the required fashion (e.g., Cope, 1973). Finally, magnetoreception is sometimes known to occur under conditions where special requirements of the hypotheses are not met (Quinn *et al.*, 1981).

We do not intend to dismiss this theoretical work prematurely. However, the difficulties with these hypothesized transduction mechanisms summarized above suggest that the older and simpler ferromagnetic transduction hypothesis warrants further examination.

The possibility that the force exerted on magnetic particles could be transduced to the nervous system has been independently suggested by Ising (1945), Lowenstam (1962), and Keeton (1972). Support for the idea came with the discovery of magnetite in magnetotactic bacteria (Frankel *et al.*, 1979; Frankel and Blakemore, 1980), and in bees (Gould *et al.*, 1978), birds (Walcott *et al.*, 1979; Presti and Pettigrew, 1980), and mammals (Zoeger *et al.*, 1981). Theoretical analyses (Yorke, 1979, 1981; Kirschvink and Gould, 1981) show that where the magnetite is present in a suitable form and in sufficient quantities, it could provide the basis for a very sensitive magnetoreceptor system capable of deriving information about direction and intensity of the geomagnetic field. Important predictions of these analyses are (1) that the magnetoreceptors should not be dependent on special conditions other than the presence of a magnetic field to function (Yorke, 1981), (2) that there should be separate compass and intensity receptors (Kirschvink and Walker, this volume), and (3) that compass accuracy and threshold sensitivity to intensity changes should be constrained by the physical properties of the magnetite crystals (Kirschvink and Walker, this volume).

The primary tests of the magnetite-based magnetoreception hypothesis must be behavioral and physiological. However, there are physical tests described in this chapter that bear on the structure and function of a magnetite-based magnetoreceptor. Such tests include magnetometry experiments designed to identify deposits of magnetite within the bodies of different organisms, and to analyze the size, shape, and arrangement of the crystals. Extraction and analysis of diffraction spectra of the magnetic material provide us with a second means of identifying the mineral. Extraction also provides measurements of the size and shape of individual crystals and information about the biomineralization process.

A feature of these studies is the very small amounts of material involved and the ease with which contaminants can affect the results of experiments (Quinn *et al.*, 1981; Jones and MacFadden, 1982; Walker *et al.*, this volume). Thus, it is important in testing for the presence of magnetite in biologic samples to test for the presence of contaminants wherever possible. Methods for doing this in whole tissues include testing for the effect of vigorous washing and ultrasonic cleaning on the magnetic properties of samples used in magnetometry experiments (Jones and MacFadden, 1982) and testing for the magnetic properties of ferromagnetic contaminants (Kirschvink *et al.*, 1985; Walker *et al.*, this volume). Assaying for trace elements associated with geologic and artificial ferromagnetic materials and examining the morphology of isolated crystals provide further means of determining the origin of the crystals.

This paper reports results of our studies of magnetoreception and its possible transduction mechanism in the yellowfin tuna, *Thunnus albacares*, and other pelagic fish. We obtained reproducible responses to earth-strength magnetic fields in the yellowfin tuna using an orthodox discrimination training procedure (Woodard and Bitterman, 1974). Using a variety of magnetometric and mineralogic techniques, we have detected, extracted, and characterized magnetite within the yellowfin tuna and other pelagic fishes. Although these studies are not as complete as we would desire, they do permit us to compare results between species from different orders, providing us with a more general insight into the putative magnetoreceptor system of fish. We then consider the results in relation to magnetoreception and magnetite biomineralization.

2. Magnetic Sensitivity in Yellowfin Tuna

The first hypothesis to test in magnetic field conditioning experiments is that animals can distinguish between different magnetic field stimuli. The procedures chosen should

therefore be appropriate to measure simple distinguishability of different magnetic fields. Decisions are required on experimental situation, testing procedure, response measure, response produced, and stimuli to be distinguished. Once it is established that the stimuli are distinguishable, different hypotheses will dictate different choices among these components of learning experiments (Kling, 1971).

Learning is detected as a relatively permanent change in behavior resulting from conditions of practice (Kling, 1971). Thus, in discrimination learning experiments, some measurable bit of behavior is modified by experience gained by the subjects during testing. In unitary, or go-no go, procedures, a single, generalized response is defined and then either positively or negatively reinforced under different stimulus conditions. Discrimination learning is then measured by comparing the readiness with which the response is expressed between the stimulus conditions (Bitterman, 1966). In choice procedures, two discrete responses that cannot be produced together are defined. In one stimulus condition, one of the responses is rewarded and the other punished. In the alternate stimulus condition, the consequences of the two responses are reversed. Discrimination is detected from the choices the animal makes between the alternate responses under the different stimulus conditions (Bitterman, 1966).

The approaches used to study magnetic field conditioning in animals can be summarized as follows. Fields were uniform and movement was restricted, limiting the subject's ability to sample the magnetic field environment (Meyer and Lambe, 1966; Reille, 1968; Kreithen and Keeton, 1974). Magnetic field polarity was the most commonly used discriminative stimulus (Phillips, 1977; Kalmijn, 1978; Griffin, 1982). The subject animal was usually required to make a choice between two alternate responses and, with one exception (Meyer and Lambe, 1966), multiple responding was not required (Bookman, 1977; Kalmijn, 1978; Griffin, 1982).

Magnetic fields are pervasive stimuli that can only be presented singly—first one, then the other—in experimental situations. Choice conditioning experiments using stimuli, such as magnetic fields, that can only be presented singly are among the most difficult discrimination problems that can be presented to experimental animals (Mackintosh, 1974) and will often fail with well-understood, salient stimuli (Bitterman, 1979). In choice procedures, trials most commonly end after the first response by the subject (e.g., Bookman, 1977; Kalmijn, 1978). However, discrimination will be sharpened if subjects are required to produce multiple responses (Bitterman, 1979), as often occurs in unitary procedures. Unitary discriminative training procedures are therefore more appropriate than choice procedures for use with magnetic fields. They should permit the subjects freedom of movement (Kreithen and Keeton, 1974), and sufficient time to sample the magnetic field environment and to produce multiple responses during trials.

For conditioning yellowfin tuna to magnetic fields, we defined a single response, required the fish to produce that response more than once, rewarded that response under one set of magnetic field conditions and not under another, and compared the readiness with which the response was expressed under the two conditions. The fish were required to swim through a 60 × 30-cm pipe frame lowered into their tank for 30-sec trial periods and retracted for intertrial periods. Within the trial periods the fish were able to swim repeatedly through the frame. Thus, the measure of behavior compared between the stimulus conditions was the rate of performance of the conditioned response. The primary advantage of rate as a measure of discrimination is its sensitivity: it can vary widely and rapidly in response to changes in experimental conditions and can accommodate short-term variability in behavior (Kling, 1971).

These experiments were conducted at the Kewalo Research Facility of the Southwest Fisheries Center Honolulu Laboratory, National Marine Fisheries Service. The fish used were captive juvenile yellowfin tuna (40- to 50-cm fork length) tested individually in one of two cylindrical test tanks (6-m diameter, 0.75-m depth). The experimental tanks con-

tained no metal and each had 100 turns of No. 18 AWG magnet wire wrapped around its perimeter. A 1-A direct current passed through these wires added a vertical magnetic field to the background field. This field was nonuniform, adding from 10 μT in the center to 50 μT at the edge of each tank. The pipe frame, the magnetic field, and a semiautomatic feeder mounted at the side of each tank (Jemison et al., 1982) were operated by mechanical and electrical linkages from an experimental control room. The control room was physically isolated from the experimental tank and was darkened during experiments. The fish were observed through small viewing ports, and their responses were recorded manually.

The differences between the two magnetic fields used in the discrimination experiments were as follows. The local Hawaiian field was uniform throughout the tanks. That is, inclination, declination, and total field intensity were the same at any point in the area occupied by the fish. The altered field introduced significant radially-oriented gradients of both intensity and inclination within the tanks. These experiments therefore provided the fish with two very different magnetic fields as discriminative stimuli. The fish could conceivably monitor differences in magnetic field inclination, intensity, or the gradients in these two parameters to make the discrimination.

In discrimination testing, a trial began with simultaneous presentation of the pipe frame and either the reinforced (S^+) or nonreinforced (S^-) stimulus. All responses by the fish within each 30-sec trial period were counted. In S^+ trials the fish was rewarded with a piece of food immediately following the first response after 30 sec. In S^- trials, a 10-sec penalty timer started at the end of the trial period. The timer was reset by each subsequent response by the fish until either the penalty time elapsed or a total of 30 sec of penalty had accumulated. Thus, response to S^- was penalized by extending the trial without any possibility of the fish obtaining food. The fish were given 30-trial training sessions held once daily. In any trial session the S^+ and S^- were presented 15 times, with no more than three S^+ or S^- trials in succession. Testing was balanced by training two fish with the normal Hawaiian field and two with the altered field as S^+.

Discrimination between the two magnetic field conditions became evident after two 30-trial sessions (Fig. 1). During the first day of testing, the response rates to the two stimuli exhibited by the fish fluctuated randomly about each other. By the end of the second day, the fish began to show a higher rate of response to the reinforced than to the nonreinforced stimulus. Some individuals showed higher response rates during nonreinforced trials just before beginning to make the discrimination. However, all individuals showed consistently higher rates of response to S^+ than to S^- on the third and fourth days of training, suggesting that they were correctly anticipating the onset of positive or nonreinforcement.

All the fish completed at least 120 trials over four or more days of testing. An analysis of variance comparing S^+ and S^- response rates over 120 trials divided into blocks of before and after 60 trials (the first two and the third and fourth training sessions) yielded an $F_{1,3}$ stimuli $= 7.61$, $p = 0.07$ and an $F_{1,3}$ stimuli by blocks $= 102.55$, $p = 0.002$. All other comparisons within the analysis did not approach significance. The main effect (stimuli) failed to be statistically significant. This is attributed to the small differences in response rates between S^+ and S^- and to the variability of responding compared with the mean rate of response. Dividing the data into blocks demonstrated that the behavior of the fish on the third and fourth days of testing was different from their behavior over the first 2 days, and that the change in behavior that occurred was dependent on the discriminative stimuli. Thus, the behavior of the fish changed as a result of their learning that production of the response had different outcomes under different magnetic field conditions.

To test whether the fish were responding to possible equipment- or observer-related cues, control trials were conducted with one fish. One of the wires connecting the power supply to the coil around the tank was disconnected and all other procedures followed as before. The response rates during reinforced and nonreinforced trials fluctuated randomly about each other during this period (Fig. 2). When the circuit between the power supply

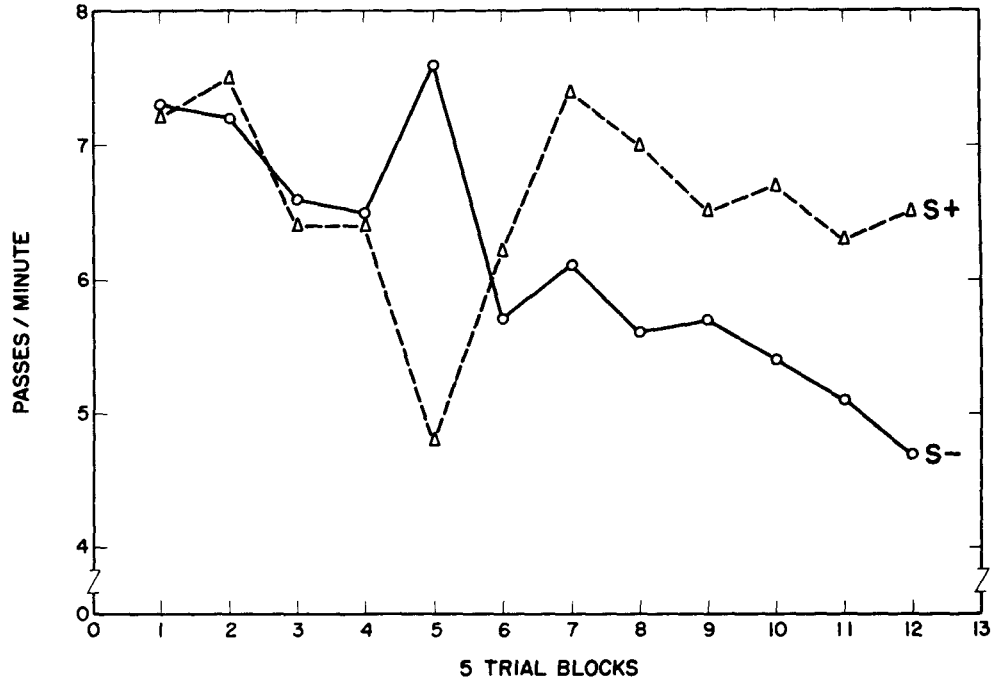

Figure 1. Magnetic field discrimination learning in yellowfin tuna. Each point is the average of five S^+ or S^- trials for the four fish tested. Data from Walker (1984).

and the coil was reestablished, the fish was again able to anticipate positive and nonreinforcement and respond accordingly (Fig. 2). However, the separation between response rates was less than before the control trials were conducted. In subsequent experiments run using double-blind procedures, two fish were easily able to discriminate between the two magnetic fields (Walker, 1984). From these results we conclude that the fish used only the magnetic fields presented to anticipate the onset of positive or nonreinforcement.

3. Detection of Magnetic Material in Fish

As noted above, to act in magnetoreception, the energy of interaction between magnetite particles and the geomagnetic field must bring about the orderly displacement of the potential of a receptor cell membrane. Kirschvink and Walker (this volume) assume that, at the receptor level, energetic considerations are of primary importance and show that single-domain particles are best suited for use in magnetite-based magnetoreception by animals. To transduce the energy of their interaction with the geomagnetic field to the nervous system, the particles must be at least partly free to move. They will then be aligned by an external field and their position or movement will transform magnetic field stimuli to mechanical stimuli (Kirschvink and Walker, this volume).

Freedom of the magnetite crystals in magnetoreceptor organelles to rotate and their small mass mean that, in the absence of an external field, their orientation will be ran-

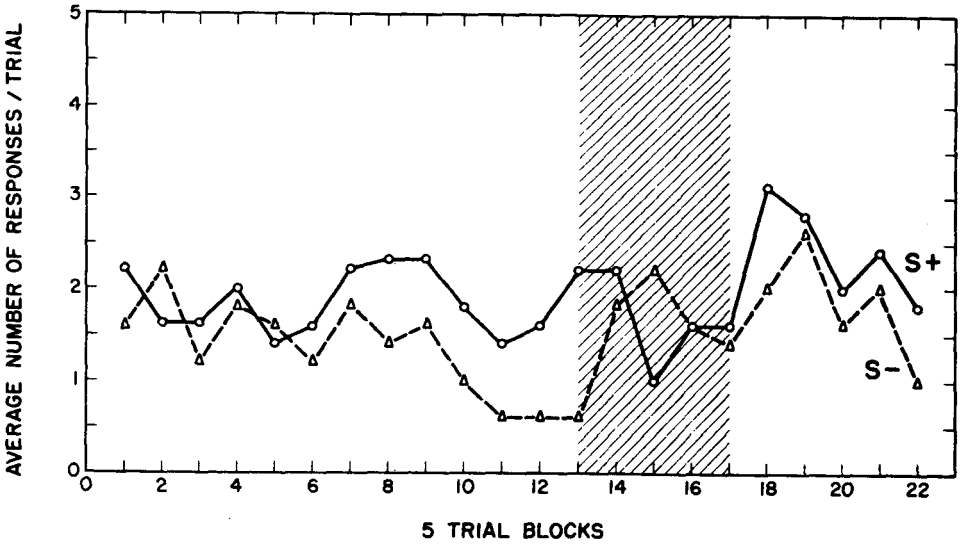

Figure 2. Magnetic field discrimination learning in individual yellowfin tuna. Shaded region in plot indicates control tests. Data from Walker (1984).

domized by thermal agitation. The particle moments will therefore cancel and not be detectable. Consequently, magnetite-based magnetoreceptor organelles will exhibit no natural remanent magnetization (NRM) in the null field environment of a superconducting magnetometer. Freezing biologic samples prevents any magnetic particles present from moving. Their moments can be realigned by momentary exposure to a strong magnetic field (>0.3 T) and will then sum to produce a net moment, the saturation isothermal remanent magnetization (sIRM).

The sIRM has been the basic sample measurement in our biomagnetic studies. Presence of magnetic material in a sample will be demonstrated by a ratio of the signal from the magnetized sample to the background signal in the magnetometer, designated the signal-to-noise (S/N) ratio, greater than unity. A scaling effect on the S/N ratio can result from sample size: Large samples are more likely than small samples to have large S/N ratios. To take account of this effect, we determined relative concentrations of magnetic material, or intensity of magnetization, in different samples by dividing their moments by their mass. In this case a reverse scaling effect could occur. Small samples with moments within or close to the background noise in the magnetometer may appear more intensely magnetized than larger samples with high S/N ratios. Determining whether or not a sample is magnetic using either of these measures alone is arbitrary. Consequently, we chose to recognize as magnetic only samples which gave both high S/N ratios and intensities of magnetization.

To distinguish magnetic material with a possible magnetoreceptive function from other deposits, we sought to identify a tissue having the following characteristics: (1) It should have a high remanent magnetic moment (or high S/N ratio) concentrated in a small volume of sample (indicated by high intensity of magnetization) compared with other tissues from the same fish; (2) the anatomic position of the magnetic tissue must be consistent from fish to fish; and (3) the bulk magnetic properties, including particle coercivity, should be similar in different individuals and in different species of fish.

Our first studies set out to determine whether magnetic material is consistently localized at any point in the body of the yellowfin tuna. Tissue and organ samples, including

Table I. Saturation Moments, Signal-to-Noise Ratios, and Intensities of Magnetization for Tissue and Organ Samples from Different Yellowfin Tuna[a]

Sample	Mean moment (pAm^2) ± S.D. (*N*)	Signal/noise	Intensity (pT) ± S.D. (*N*)
Liver	105.0 ± 134.4 (2)	2.1	1.8
Pyloric cecum	49.6 ± 50.0 (3)	1.0	3.5
Intestine	14.5 ± 20.5 (2)	0.3	4.8
Red muscle	184.0 ± 274.7 (3)	3.7	3.5
White muscle	155.0 ± 211.2 (6)	3.1	5.7 ± 5.3 (3)
Brain	36.4 ± 50.3 (5)	0.7	7.5
Gill	95.0 ± 143.4 (6)	1.9	20.6
Skin	41.7 ± 80.1 (6)	0.8	35.7
Peduncle tendon	120.0 ± 169.7 (2)	2.4	41.4
Frontal bone	202.0 ± 129.0 (6)	4.0	103.6 ± 86.9 (2)
Pectoral fin	325.0 ± 427.0 (2)	6.5	62.5
Posterior brain case	375.0 ± 455.0 (6)	7.5	ND
Dorsal fin	400.0 ± 628.5 (5)	8.0	ND
Cardiac muscle	500.0 ± 707.1 (2)	10.0	4.5
Eye	1242.5 ± 1052.6 (4)	24.9	13.7 ± 14.1 (2)
Dermethmoid bone	1320.6 ± 867.5 (15)	26.4	127.0 ± 86.7 (7)

[a] Variability estimates are standard deviations and numbers in parentheses are the number of samples measured for the saturation moments and intensities of magnetization. Intensity estimates for many samples came from one fish only; "ND" indicates no data. Signal-to-noise ratios were calculated by dividing the mean saturation moment by the maximum background noise (50 pAm^2) in the magnetometer. Samples are grouped into those which were clearly nonmagnetic, those which were magnetic from their signal-to-noise ratio or their intensity of magnetization only, and those which were magnetic from both measures.

bones of the body and skull, skin, sense organs, viscera, and swimming muscles, were dissected from three fish (fork length 40–50 cm) with glass microtome knives and handled with nonmetallic tools in a magnetically shielded, dust-free, clean room. Although subsequent dissections focused on the 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, and tested for IRM in a superconducting magnetometer. Techniques for laboratory preparation and dissection of tissue samples have been described elsewhere (Kirschvink, 1983; Walker *et al.*, this volume).

Six tissue or organ samples showed neither high S/N ratios nor high intensities of magnetization (Table I). Seven other samples had moments less than 10 times the background noise in the magnetometer. However, because of the scaling effect caused by their small mass, these samples showed high intensities of magnetization. Cardiac muscle and eye samples acquired high moments but had relatively low intensities of magnetization. Subdivision and remeasurement showed that the moments acquired by eye samples were not associated with the lens, retina, or optic nerve. Because all these tissue samples were either clearly nonmagnetic, appeared magnetic from one measure only, or were not reproducible in different fish, it seemed unlikely that they would come from a sensory organ; for this reason we focused our work on tissues that acquired large, reproducible moments.

Only the dermethmoid bone gave high values for all measures of magnetization used in all fish examined (Table I). A scatter diagram, plotting intensity of magnetization against S/N ratio for the data presented in Table I, clearly identified the dermethmoid bone as the most magnetic sample measured. Subdivision and remeasurement of the dermethmoids

Table II. Saturation Isothermal Remanent Magnetization in Selected Tissues of Different Fish[a]

Species	Number sampled	Tissue IRM (pAm^2)		
		Dermethmoid	Muscle	Eye
Thunnus albacares	16	260–3000	20–500	100–2600
T. alalunga	2	100–200	30	1700
T. obesus	1	480	400	ND[b]
Sarda orientalis	1	1500	700–1200	5000
Scomber sp.	1	1750	60	90
Makaira nigricans	4	110–170	ND	ND
Oncorhynchus tshawytscha[c]	4	310–360	50–73	100–2300
Engraulis mordax	5	235–2850	40–170	ND

[a] Background signal in magnetometer 10–50 pAm^2.
[b] ND, no data.
[c] Olfactory rosette and brain always nonmagnetic in *O. tshawytscha*; other tissues, especially viscera, variable in all species.

in a number of fish suggested that the magnetic material was contained in tissue in a sinus formed within the bone.

Taken alone, the S/N ratio data could lead to the conclusion that many tissues are magnetic in the yellowfin tuna. However, most samples were only occasionally magnetic and often vigorous washing or ultrasonic cleaning would reduce the IRM acquired by such samples. This suggests the presence of external contaminants in samples where the cleaning procedure reduced the moment and anomalous deposits of magnetic material where it did not.

We then tested for the presence of magnetic material in the bodies of other pelagic fish species. We chose to ignore many of the corresponding tissues that were nonmagnetic in the yellowfin tuna, and concentrated instead on those associated with the head. As in the yellowfin tuna, these experiments did not identify or characterize the magnetic material but they did allow us to (1) identify tissues that were magnetic in all individuals, (2) identify tissues that were probably magnetic due to the presence of contaminants, and (3) identify areas that may have been magnetic due to the presence of anomalous deposits of magnetic material.

Fish from the orders Perciformes (families Scombridae, Istiophoridae, Coryphaenidae), Clupeiformes (family Engraulidae), and Salmoniformes (family Salmonidae), all had magnetic material associated with the dermethmoid bone or the anterior region of the skull (Table II). The sIRM values ranged between 100 and 3000 pAm^2 for dermethmoid bones from nine species of pelagic fish. Most of these sIRM values fell in the range from 100 to 1000 pAm^2, being most consistent among individuals for the blue marlin, *Makaira nigricans*, and chinook salmon, *Oncorhynchus tshawytscha*.

In our survey of different species, we worked mostly with the heads of fish. These heads had often been cut with either a metal saw or a knife, which could easily have contaminated tissues in the region of the cuts (see Bauer *et al.*, this volume). Where we were able to work with whole fish, only tissues in contact with the environment frequently acquired magnetic moments (Table II). For example, the northern anchovy, *Engraulis mordax*, we studied were obtained whole from the Southwest Fisheries Center La Jolla Laboratory, National Marine Fisheries Service. Their gills and guts frequently acquired moments. Vigorous rinsing with distilled water sometimes led to loss of the sIRM acquired by these tissues. Muscle samples, which could not have been exposed to the environment, were not magnetic in any of the anchovies we examined. Magnetic muscle samples in the

heads of two chinook salmon could have been contaminated by knife cuts but did not lose their moments after cleaning. The moments acquired by these samples may therefore be due to biochemical deposits of magnetic material, and a number of tests will be necessary to characterize them. Walker *et al.* (this volume) describe these tests and discuss the analysis of a tissue contaminated by saw cuts. Here we focus on tests conducted on the dermethmoid tissue in a number of species.

4. Characterization of the Magnetic Material

Studies of the acquisition and loss of magnetization by samples permitted us to identify tentatively the source of the sIRM in the dermethmoid bone of the yellowfin tuna and to make inferences on the organization of the magnetic crystals. A prediction of the magnetite-based magnetoreception hypothesis is that the particles are free to move and that their orientation will be randomized by thermal agitation at room temperature in a null field environment. If the dermethmoid bone is the site of magnetoreceptor organelles, it should possess no NRM and its acquired moment should be lost if it is allowed to thaw. The frozen dermethmoid bones of seven yellowfin tuna first were tested for NRM. We magnetized these samples, allowed them to warm to room temperature, and measured their moments at 5 min-intervals as they thawed. Four of these samples were then washed, refrozen, and subjected to progressively increasing magnetic fields in an impulse magnetizer (Kirschvink,

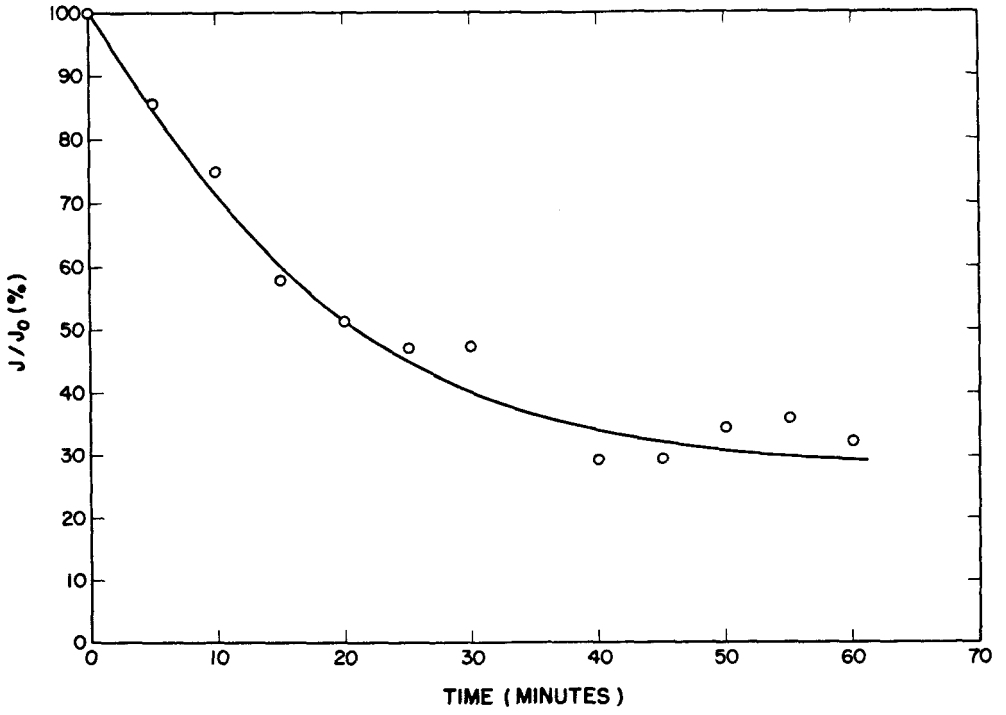

Figure 3. Loss of remanent magnetization with time on warming from liquid nitrogen temperature (77°K) to room temperature (293°K) in the dermethmoid tissues of seven yellowfin tuna. Only two measurements were taken at 35 min.

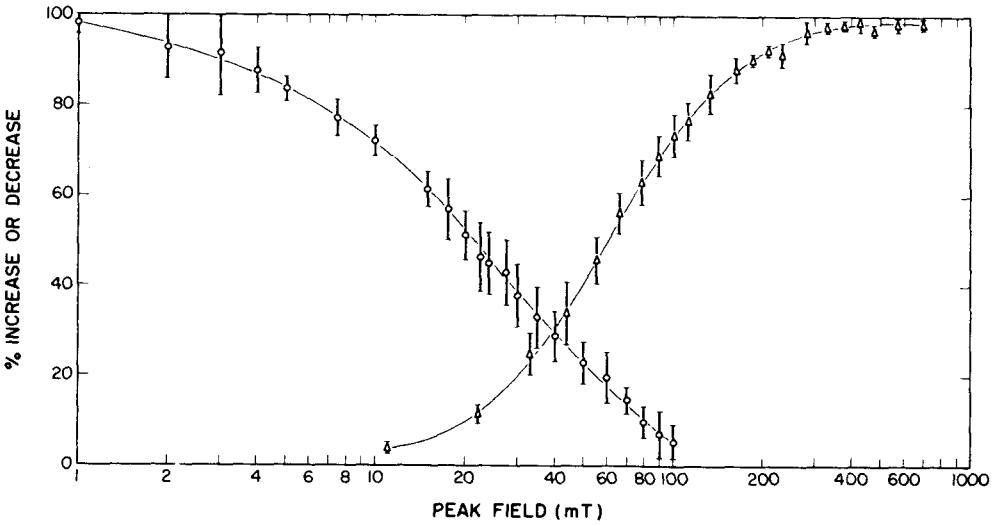

Figure 4. Progressive acquisition and loss of remanent magnetization by the dermethmoid tissues of four yellowfin tuna. Vertical bars indicate standard errors. The ordinate and abscissa of the intersection point are approximately 30% (R = 0.3) and 40 mT, respectively (see text). From Walker *et al.* (1984).

1983). This procedure was followed by progressive alternating field (AF) demagnetization of the samples after they were saturated. After each step in these magnetization and demagnetization experiments, we remeasured the moments of the samples.

The frozen dermethmoid bones of the yellowfin tuna showed no NRM (moments 3–30 pA m^2). After saturation, all showed an exponential decay with time in the moments they retained (Fig. 3). This suggests that the orientation of the crystals was randomized by thermal agitation as the tissue thawed. From this observation we conclude that the crystals are at least partly free to rotate.

The dermethmoid tissues of four yellowfin tuna acquired virtually all of their remanence in fields less than 200 mT and lost it again in unblocking fields between 10 and 100 mT (Fig. 4). The relatively narrow range of fields over which the dermethmoid tissues acquired and lost remanent magnetization is consistent with the acquired moments resulting from single-domain crystals of magnetite. However, the estimates of median coercivity of the magnetic particles obtained from the two methods differed significantly. This discrepancy results from interactions between the magnetic particles.

The AF demagnetization curve for a dispersion of noninteracting single-domain crystals should be symmetrical about the 50% level with the IRM acquisition curve. Asymmetry in the two curves implies that the neighboring particles are sufficiently close for their magnetic moments to interact to aid AF demagnetization and inhibit IRM acquisition (Cisowski, 1981). These interaction effects lead to under- and overestimates of the median microscopic coercivities of the crystals, respectively. Cisowski (1981) found that the shift toward higher coercive fields in the IRM curve, and the shift to lower coercive fields in the AF demagnetization curve are almost exactly the same. As a result, the abscissa of the intersection point is independent of the interaction effect and provides an estimate of the remanent coercive field. This has a value of 40 mT for the crystals in the yellowfin tuna dermethmoid tissue. The crystals therefore fit into the single-domain magnetite region with particle lengths of approximately 50 nm and axial ratios of about 0.8 in the Butler–Banerjee diagram (see Fig. 4 in Kirschvink and Walker, this volume). Depending on the size dis-

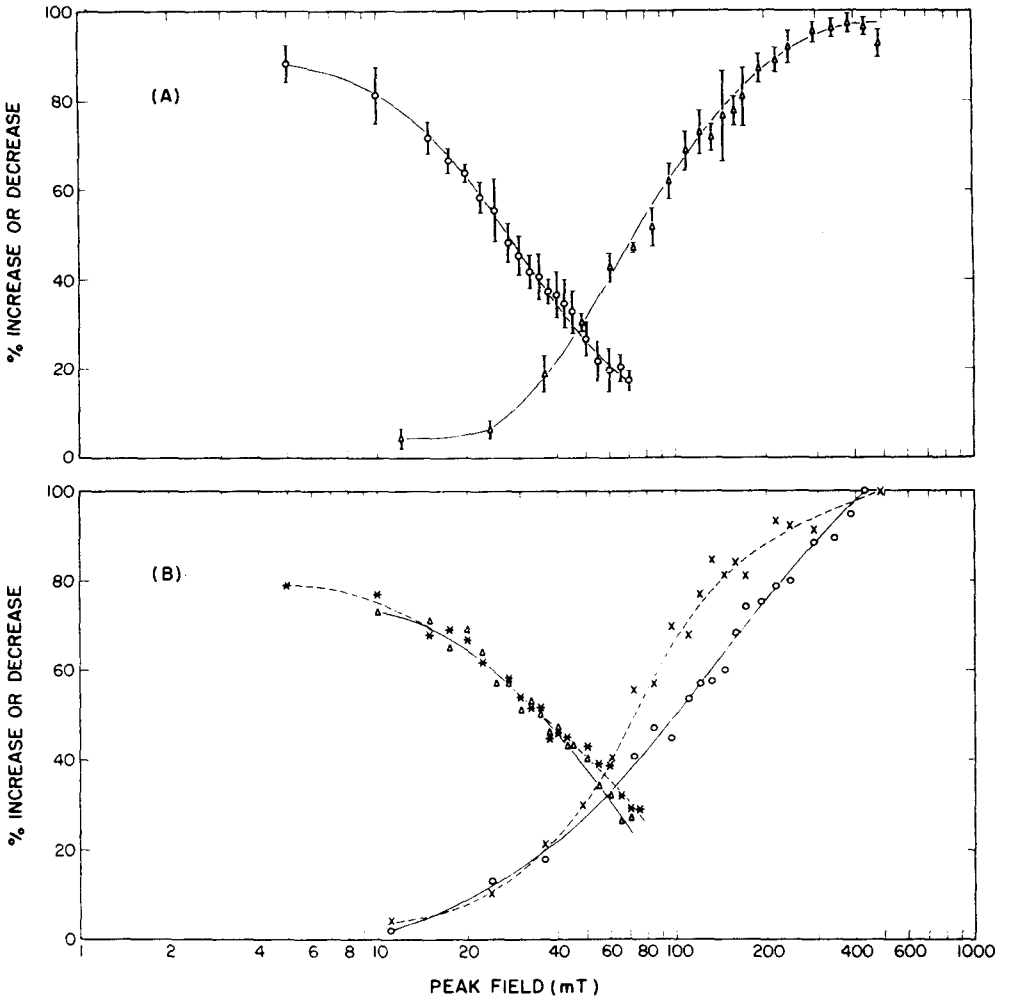

Figure 5. Progressive acquisition and loss of remanent magnetization by the dermethmoid bones of (A) four chinook salmon and (B) one chub mackerel (dashed lines) and one striped bonito (solid lines). Vertical bars in (A) indicate standard errors. *R* values are 0.32 (chinook salmon), 0.37 (chub mackerel), and 0.32 (striped bonito). Estimates of median coercivities of the magnetic particles taken from the abscissae of the intersection points are 46 mT (chinook salmon), 58 mT (chub mackerel), and 60 mT (striped bonito).

tribution of particles, between 1 million and 100 million crystals would be necessary to produce the sIRMs observed. These numbers are comparable to the estimates of the number of single-domain crystals possessed by honeybees (Gould *et al.*, 1978) and homing pigeons (Walcott *et al.*, 1979).

The intersection of the AF demagnetization and IRM acquisition curves for the tuna dermethmoids is at approximately 30% magnetization [or $R = 0.3$ as discussed by Cisowski (1981)] (Fig. 4). This is well below the value of 0.5 expected for completely noninteracting single-domains. The crystals of magnetite in the dermethmoid tissue of the yellowfin tuna

therefore interact significantly and are about as closely associated as the crystals in the semidispersed powder ($R = 0.3$) studied by Cisowski (1981).

Comparative data are available for a number of species. The IRM acquisition and AF demagnetization curves (Fig. 5) for dermethmoid samples from individual chub mackerel, *Scomber japonicus*, and striped bonito, *Sarda orientalis*, and for four chinook salmon are almost identical in form to those obtained for the yellowfin tuna. The AF demagnetization curves for the dermethmoids of two blue marlin were also very similar to the other AF demagnetization curves and gave a median coercivity estimate of 18 mT (Walker, unpublished data). These results give particle length estimates of 50–60 nm and axial ratios of 0.5–0.8 for magnetite particles in all these fish. The consistency of the results in species of very different sizes and from different orders argues against the possibility that their dermethmoids contain contaminants. We have carried out a number of tests which conclusively exclude contaminants as the source of remanence in the dermethmoid tissue of the yellowfin tuna and which further demonstrate the high degree of control exercised over the deposition of the magnetic particles.

The IRM acquisition and AF demagnetization curves for yellowfin tuna, chinook salmon, striped bonito, and chub mackerel are very flat at fields less than 10 mT and also at fields greater than 200 mT. This excludes multidomain magnetites, commonly found in igneous rocks and laboratory dust, as the source of remanence in the dermethmoid tissues. Multidomain magnetites are magnetically soft (Kirschvink, 1983) and acquire or lose magnetization in much lower fields than did the dermethmoid tissues of these pelagic fishes. The flattening of the IRM acquisition curves above 200 mT excludes hematite and many iron alloys, which will continue to acquire remanence in fields above 1 T. Therefore, we can exclude almost all ferromagnetic minerals except maghemite and synthetic magnetite as the source of remanence in the dermethmoid tissues of pelagic fishes. The extraction and analyses of the magnetic particles discussed next enable us to exclude even these possible sources of magnetic remanence.

5. Identification and Analysis of the Magnetic Material

The extraction techniques discussed by Walker *et al.* (this volume) permitted a number of distinctive assays for magnetite in the tuna dermethmoid tissue. Magnetic particles extracted from the tissue were black, both to the naked eye and when viewed under a dissecting microscope. This excluded maghemite as a possible source of the remanence in the dermethmoid tissue and strongly suggested that the only magnetic mineral present was magnetite. In an attempt to determine whether normally nonmagnetic tissues also contained finely dispersed magnetic material, a large sample (about 10 g) of the white muscle of one fish was digested using the same techniques. No magnetic particles were obtained, presumably because any particles present in the swimming muscle must have been present in concentrations too small to be extracted using these techniques.

X-ray diffraction, the technique used to identify the crystals, depends on the interaction between the collimated X-ray beam, the ions in the mineral, and their orientation in the crystal lattice. The beam enters the sample and is scattered at angles characteristic of the position of each ion in the lattice. The scattered beam is detected by X-ray photographic film which, after development, shows a series of concentric arcs. The distance of each arc from the center is thus characteristic of the structure and composition of the sample crystals. From these distances are calculated the distances (known as d spacings) between adjacent ions in the unit cell of the lattice.

X-ray diffraction of the magnetic material extracted from the dermethmoid tissue of the yellowfin tuna uniquely identified magnetite as the source of remanence (Fig. 6). The

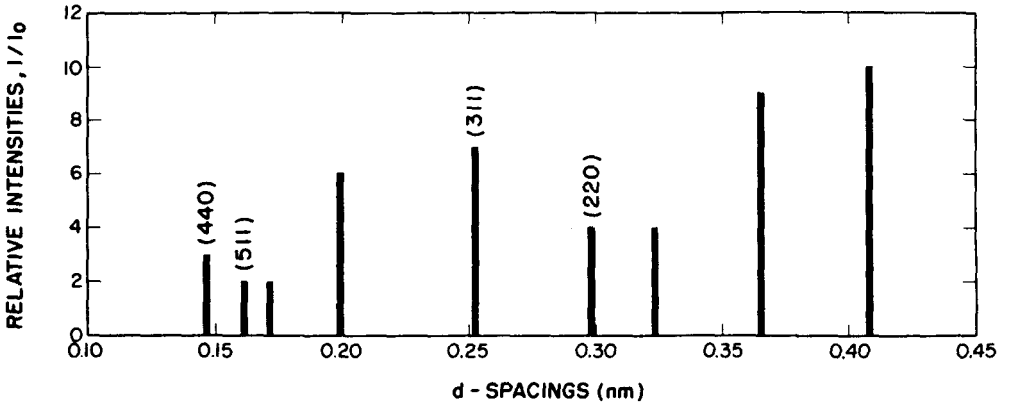

Figure 6. X-ray diffraction data for magnetite extracted from tissue contained within the dermethmoid bone sinus of the yellowfin tuna. Vertical lines indicate relative intensities of lines in the diffraction pattern. Numbers in parentheses indicate lines associated with magnetite and the crystal axis giving rise to each line.

lattice parameter estimated from X-ray diffraction is 0.8358 ± 0.004 nm (reference value 0.8396 nm). The origin of the lines not associated with magnetite in the pattern is unknown, although they do not arise from any known ferromagnetic mineral. Two possible sources of these lines are connective tissue associated with the crystal aggregate and insoluble proteins involved in an organic matrix in which the crystals are deposited (Weiner *et al.*, 1983).

Electron microprobe analysis of aggregated crystals showed that the magnetite from the yellowfin tuna is remarkably pure. The crystals contained no measurable titanium and almost no manganese (Table III), which are common components of geologic magnetites. The crystals also contained no measurable chromium, excluding many possible synthetic ferromagnetic minerals. As in the magnetometry studies, we can thus rule out almost all nonbiologic origins for the magnetite crystals found in the tuna.

We were able to use transmission electron microscope (TEM) studies to measure the size and shape of the isolated magnetite particles. These crystals averaged 45 nm in length, 38 nm in diameter, and had a subcubic form (Fig. 7). The crystals are thus single-domains and conform to the size and axial ratio ranges predicted from their coercivities (see above).

Table III. Electron Microprobe Analyses of Magnetite Particles isolated from Yellowfin Tuna

Oxide	Magnetite standard (NMNH 11487)	Weight (%) of sample
FeO	90.9	86.3 + 7.7
TiO_2	0.2	0.0 ± 0.0
Cr_2O_3	<0.25	0.0 ± 0.0
MnO	<0.0	0.2 ± 0.1
CaO	—	0.2 ± 0.0
Total	91.4	86.7

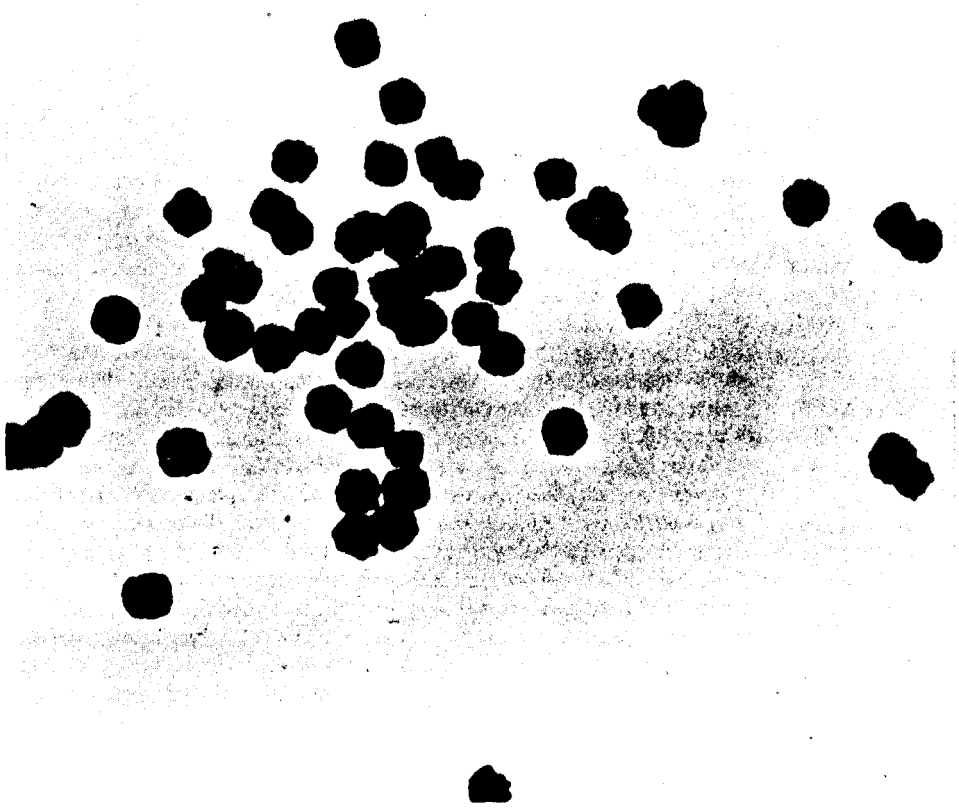

Figure 7. Free magnetite grains extracted from the dermethmoid tissue of the yellowfin tuna. Transmission electron micrograph courtesy of R. S.-B. Chang. From Walker *et al.* (1984).

Their morphology differs from the octahedral crystal form found in all nonbiogenic magnetites. On the basis of this crystal form, we conclude that these crystals could not have come from nonbiologic sources, but must have a biogenic origin. This is consistent with the results obtained from the different approaches used in this study. The evidence from a variety of methods for biogenic origin of the crystals gives us greater confidence when discussing our results and their implications for magnetoreception and magnetite biomineralization in fish.

6. Discussion

There is a growing body of evidence that fish respond to magnetic fields. Elasmobranch (Kalmijn, 1978) and teleost (this study) fish have been conditioned to respond to magnetic field stimuli while salmoniform (Quinn, 1980; Quinn *et al.*, 1981; Quinn and Brannon, 1982) and anguilliform (Tesch, 1974) fish have shown unconditioned directional responses to magnetic fields. Directional responses imply that fish possess a magnetic compass. The conditioning experiment reported here (Figs. 1 and 2) provided changes in magnetic field

intensity, intensity gradients, and inclination to fish being conditioned to discriminate between magnetic fields. Thus, it is possible that the fish responded to intensity or intensity gradients in these experiments. There is an important need for further conditioning experiments to test the responses of fish to these components of the geomagnetic field and to test the predictions of the ferromagnetic magnetoreception hypothesis (Kirschvink and Walker, this volume).

It has not yet been established that fish use the geomagnetic field for direction finding or navigation in the ocean. Quinn (1982) presents arguments that migrating Pacific salmon must be able to navigate to achieve the distances traveled in the time taken to return from the open ocean to the North American coast. Quinn goes on to suggest that these fish may use a compass and a magnetic map based on a bicoordinate grid of magnetic field inclination and declination for navigation.

There is some field evidence that fish are able to determine their position and swimming direction, although the sensory bases for these abilities are unknown. Short-term tracking experiments show that a number of different pelagic fishes, including swordfish, *Xiphias gladius* (Carey and Robison, 1981), and Atlantic salmon, *Salmo salar* (Smith *et al.*, 1981), maintain relatively constant compass courses for substantial periods (up to several days in the swordfish tracked by Carey and Robison). In addition, skipjack tuna (Yuen, 1970) and swordfish (Carey and Robison, 1981) make diurnal return movements to discrete areas (shallow banks) without retracing their outward path. The sensory mechanism or mechanisms responsible for guiding these movements are as yet unknown. However, the ability of fish to detect magnetic fields suggests that the possibility that they use the geomagnetic field to guide their movements is worth serious experimental investigation.

The lack of feasible transduction mechanisms to explain magnetoreception has hindered the testing of hypotheses concerning magnetic sensitivity in animals. One mechanism for transduction of magnetic field information to the nervous system has been suggested for the elasmobranchs by Kalmijn (1974, 1978). On the basis of his theoretical analysis, Kalmijn (1974) predicted that the elasmobranchs should be able to detect magnetic fields using their ampullae of Lorenzini. This prediction appeared to be borne out by his later successful conditioning of rays to respond to magnetic fields (Kalmijn, 1978). However, the critical behavioral experiment to determine whether or not electrical field information is necessary for magnetoreception in the elasmobranchs has yet to be carried out.

Discovery of single-domain magnetite in a variety of metazoan groups provides the basis for a general magnetoreception mechanism suitable for use in both aquatic and terrestrial environments. In the vertebrates, single-domain magnetite has been found in the anterior dura mater membrane or in association with the ethmoid areas of the skull (Baker, Chapter 26; this volume; Bauer *et al.*, this volume; Mather, this volume; Perry *et al.*, this volume). We have found single-domain magnetite with virtually identical magnetic properties in the dermethmoid tissue of representatives of different orders of teleost fish. Our studies of the magnetite crystals in the yellowfin tuna showed that the crystals average 45 nm in length by 38 nm in diameter (Fig. 7) and are arranged in interacting arrays that appear at least partly free to rotate. These results enable us to refine our predictions concerning magnetoreceptor organization and sensitivity in fish.

The magnetic properties of the magnetite crystals will determine and constrain the operation of magnetite-based magnetoreceptor organelles (Kirschvink and Walker, this volume). From the dimensions of the crystals extracted from the yellowfin tuna, we can calculate that 8.5×10^7 particles are necessary to produce the mean remanence observed in the dermethmoid tissue. The energy of interaction of the individual crystals with the geomagnetic field will be about 0.1 kT (see Fig. 4 in Kirschvink and Walker, this volume). To achieve coupling energies with the geomagnetic field large enough for detection by the nervous system, the crystals must therefore be organized into interacting arrays. Depending

on the numbers of crystals in the arrays, the maximum theoretical sensitivity of a magnetite-based magnetoreception system in fish can easily be estimated (Yorke, 1981). The apparent freedom of the crystal arrays to rotate suggests that a mechanoreceptor monitoring position or movement of the arrays is a suitable means to link the magnetite crystals to the nervous system.

The appeal of the ferromagnetic magnetoreception hypothesis is that it can theoretically account for the responses to magnetic fields exhibited in such diverse groups as the bacteria (Frankel and Blakemore, 1980), algae (Lins de Barros *et al.*, 1981; Lins de Barros and Esquivel, this volume), bees (Lindauer and Martin, 1972), fish (Quinn, 1980), and birds (Keeton, 1969). In the unicellular organisms and honeybees, the hypothesis has been tested experimentally (Kalmijn, 1981; Kirschvink, 1981; Frankel *et al.*, this volume). For fish, some indirect evidence for the hypothesis is also available. Quinn *et al.* (1981) state that the magnetoreceptor of the sockeye salmon must be able to function in the dark, in salt and fresh water, in the absence of water flow, and be evolutionarily adaptable to magnetic field reversals. Although not a test of the hypothesis, these behaviorally determined constraints argue against the optical pumping and electrical induction hypotheses for magnetoreception (Leask, 1977; Kalmijn, 1978) and are all compatible with possession by the salmon of a ferromagnetic magnetoreceptor (Kirschvink *et al.*, 1985).

The case for magnetite-based magnetoreception in metazoans will not be proven until linkage of the crystals to functioning sensory nerves transmitting magnetic field information to the central nervous system has been demonstrated. However, the discovery of biogenic magnetite, suitable for use in magnetoreception in different vertebrate and invertebrate organisms, cracks the conceptual nut concerning a general magnetic sensory mechanism and provides a good working hypothesis for the testing of many ideas about magnetoreception. Substantial support for the hypothesis will come from experiments that test for ferromagnetic effects on behavioral responses to magnetic fields. Kirschvink and Walker (this volume) suggest experiments to estimate the magnetic moments of magnetite-based magnetoreceptors and to test the theoretical constraints on ferromagnetic compass and intensity receptors.

The variety of techniques used in these studies provided us with a number of internal checks on our work. Through magnetometry studies we were able not only to identify those areas of the bodies of fish that were magnetic, but also to show that the sIRM acquired by the dermethmoid tissue was due to single-domain particles and not to magnetically very soft or very hard contaminants. The microprobe analyses showed that the crystals from the yellowfin tuna contained almost none of the impurities characteristic of geologic magnetites. Finally, the unique nonoctahedral morphology of the crystals compared with the octahedral crystal form of all other magnetites demonstrates the biogenic origin of the crystals seen in TEM (Fig. 7; Lowenstam and Kirschvink, this volume).

Our studies on the magnetite crystals extracted from yellowfin tuna bear on the process of magnetite biomineralization. The diffraction spectra show that the crystals are very pure, implying that they are formed under close chemical control. The crystals are also very uniform in size and shape. These properties are characteristic of biominerals formed under matrix-mediated control (Lowenstam, 1981; Lowenstam and Kirschvink, this volume).

The only well-studied examples of magnetite biomineralization are in the chitons and bacteria. These organisms appear to lay down a template of organic matrix and biochemically precipitate magnetite within it under enzymatic control (Kirschvink and Lowenstam, 1979; Balkwill *et al.*, 1980; Nesson and Lowenstam, this volume). Matrix-mediated biomineralization may be the mechanism for magnetite precipitation in fish as it could provide the means for control of the size, shape, and composition necessary for use of the crystals in magnetoreception. Locations of the crystals and their site of deposition in intact tissues could provide us with valuable understanding of both their biomineralization and their role in magnetoreception.

Biomineralization processes have a long evolutionary history. The widespread use of very similar matrix-mediated biomineralization processes in metazoans for skeleton building from the late Precambrian period (Weiner *et al.*, 1983) suggests a common origin for matrix-mediated biomineralization dating to the early Precambrian (Lowenstam and Weiner, 1983). Magnetite, presumably formed by matrix-mediated biomineralization, has now been identified in many phylogenetically distant metazoan groups. From arguments similar to those advanced by Lowenstam and Weiner (1983), two interpretations of the appearance of magnetite in these groups can now be offered. Biogenic magnetite in the metazoans arose from a long history in the Precambrian which predates the differentiation of the metazoan phyla, or it arose from multiple, independent origins in the late Precambrian, after the divergence of the metazoan phyla. Resolution of the problem of the origin of biogenic magnetite in the metazoans may depend on identification of biogenic magnetites of clear metazoan origin from before the late Precambrian.

Lowenstam and Weiner (1983) suggest that the ability to produce magnetite evolved in the early Precambrian as a means of iron storage in the reducing environment of that time. In bacteria possessing magnetite crystals, the ability to perform directed swimming responses may have provided selective advantages for magnetotaxis, even before the advent of an oxygen-rich environment. In the metazoans, it seems necessary to postulate selection operating on an association between magnetite crystals and sensory nerves as the origin of magnetoreception. This assumes that magnetite played some other, prior role in the body of the organism. Other studies have reported the presence of magnetite or magnetic material which does not have an apparent magnetoreceptive function (e.g., Presti and Pettigrew, 1980) and which may be lost in anemic individuals (Baker *et al.*, 1983). Study of these deposits and the sites and conditions under which they form may elucidate the early functions of magnetite in the metazoans.

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