

Chapter 21

Magnetoreception and Biomineralization of Magnetite in Amphibians and Reptiles

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1. Introduction	439
2. Amphibians	440
3. Reptiles	443
3.1. Magnetoreception in Sea Turtles	443
3.2. Isolation and Identification of Magnetite in Sea Turtles	447
4. Conclusion	452
References	452

1. Introduction

Many amphibians and reptiles are seasonally migratory, traveling to and from suitable breeding, feeding, or hibernation grounds. These creatures also carry out small-scale directed movements in local areas. As juveniles, they must locate appropriate areas for growth and maturation, often in environments vastly different from those into which they hatched. Thus, soon after hatching, sea turtles scurgle up through the sand and find their way down the beach to the ocean, while tadpoles, after spending several weeks in an aquatic environment, metamorphose into frogs and climb out of their ponds onto dry land. As adults, the amphibians and reptiles slither, stalk, or swim about in search of food and shelter and to escape predators.

The distances covered by amphibians and reptiles in all of these movements are, with notable exceptions, not of great extent, and in most cases the use of sight, smell, hearing, and touch are probably sufficient for direction finding. In order to orient in familiar areas while engaged in foraging, escaping from predators, or other routine movements, these animals probably depend upon reference to learned landmarks, routes, and features of topography (Ferguson, 1971). The occasional longer foray into unfamiliar territory, unusual weather or other adverse environmental conditions, and ontogenetic and seasonal migrations may, on the other hand, require the use of a host of sensory capabilities. Studies have shown that amphibians and reptiles can call on multiple sensory bases for direction

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finding (reviewed in Adler, 1982), and are capable of employing alternative cues and sensory mechanisms when circumstances dictate or when denied use of a normally functional sense.

The use of magnetic information for orientation has been demonstrated in other vertebrates, including homing pigeons (Keeton, 1971), salmon (Quinn, 1980), elasmobranchs (Kalmijn, 1978), eels (Tesch, 1974), and woodmice (Mather and Baker, 1981). Only preliminary studies, however, have looked specifically at magnetic sensitivity as a component of amphibian and reptile orientation. Magnetic cues could be of particular importance to amphibians and reptiles in environments where visual cues are limited, as is the case for two of the animals discussed below, the cave-dwelling salamander and the marine turtle.

2. Amphibians

Amphibian movements are generally restricted to distances less than a few kilometers. Within their limited range, seasonal migrations and movements around local areas are well oriented (Schmidt-Koenig, 1975), and, in displacement studies, amphibians have exhibited remarkable homing abilities. In one of many such experiments designed to examine amphibian orientation, Twitty *et al.* (1967) displaced red-bellied newts up to 8 km away from their native stream segment, either to other segments of the same stream or across a ridge to some foreign stream. Over 60% of the displaced newts were able to find their way back to their home stream segment, usually in the next breeding season. To account for such homing accuracy among amphibians, researchers have investigated the use of olfaction, vision, extraoptic receptors, and sun and celestial compasses, but have rarely questioned whether magnetoreception may also be involved.

Phillips (1977) provided evidence for a learned directional response to earth-strength magnetic fields in the cave salamander, *Eurycea lucifuga*. His objective was to determine if animals trained to move through passageways under specific magnetic field conditions would, when tested, use the learned magnetic information as an orientation cue. For training, he confined two groups of 15 salamanders each in two separate training corridors. Each corridor consisted of two compartments filled with pieces of limestone, connected by a darkened central passageway. Moisture was supplied alternately to one of the two end compartments, forcing the animals to move at intervals from one compartment to the other through the passageway. The two corridors were aligned along the same topographical axis, parallel to the earth's N-S magnetic field, with one, the "a" corridor, enclosed in a coil which rotated the magnetic field 90° clockwise, and the other, the "b" corridor, in the normal field. Movement of the A group was therefore perpendicular to the magnetic N-S axis, while that of the B group was parallel to the earth's field. For testing, the two groups were released simultaneously into the center of a cross-shaped assembly made up of the two training corridors connected perpendicularly to one another. Animals were released into the center of the testing assembly, and after 40 min the location of each salamander was recorded. Animals were tested in both the natural and the altered magnetic field, and with the cross assembly positioned with either the "a" or the "b" corridor parallel to the topographical axis along which both were aligned in training.

In 6 of 16 tests there was a significant difference between the groups in their choice of corridors, with the difference resulting from the groups moving in the direction predicted from training, that is, perpendicular to the N-S magnetic axis for the A group and parallel to the N-S magnetic axis for the B group (Fig. 1). In these cases the animals' distribution shifted in the manner predicted by the orientation of the magnetic field only. Although a directional response to magnetic fields was clear in these six tests, in the majority of tests the distribution of the two groups was not significantly different. Phillips believes this

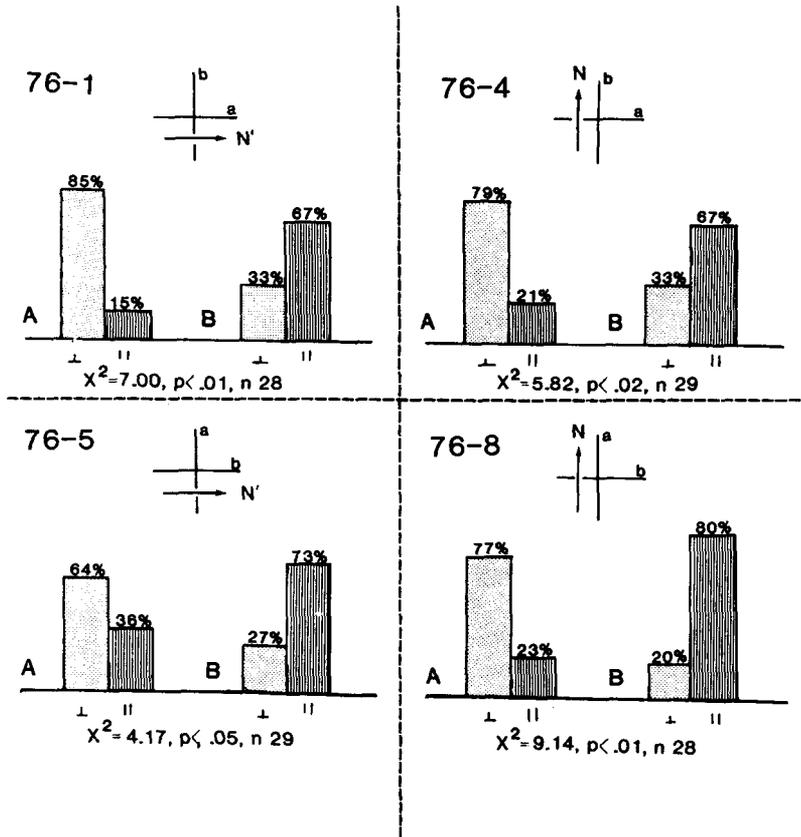


Figure 1. Results of tests with salamander groups A and B, previously trained in corridors a and b, respectively. These tests represent the four possible combinations (insets) of the expected magnetic directions relative to the home corridors and the topographical axis. The histograms show the percentage of each group of animals which moved perpendicular and parallel to the natural or altered field. For example, in test 76-1, animals in group A, which were originally trained to move perpendicularly to the magnetic field, do so preferentially (85%) even though the corridor perpendicular to the magnetic axis (b) is not their home corridor; this preferred movement also coincides with the topographical axis (represented by the vertical axis in each inset). For statistical purposes, the combined number of A- and B-group animals which moved along the expected axis is tested (chi-square, one-tailed, one degree of freedom) against the null hypothesis of a 50:50 distribution of both groups along the two perpendicular axes. From Phillips (1977).

discrepancy may have been due to different factors for each group. The A group may have been influenced by familiar vibrations, as the tests were carried out in the location where this group had been trained. Natural disturbance in the earth's magnetic field appeared to affect the B group's performance, with increased deterioration in performance correlated with the magnitude of disturbance.

Related experiments were carried out by Phillips and Adler (1978) with eastern red-spotted newts (*Notophthalmus viridescens*), to determine if newts exhibit a locomotor or positional response when placed in a magnetic gradient. Newts were individually tested by placing them in aquaria oriented along the earth's N-S magnetic axis. As in Phillips's

Table I. Mean Positions of Newts under Three Magnetic Conditions^a

Test sequence	Date	A	B	C	D	E	F	Test mean
Mag-0 tests								
1	July 14	N 18.47	N 22.07	N 22.10	N 12.12	N 15.85	N 0.84	N 15.21
4	July 18	N 9.02	S 4.75	N 1.17	N 18.87	S 2.54	1.27	N 3.43
7	July 21	N 10.43	N 18.77	<i>excluded</i>	<i>excluded</i>	N 9.22	<i>excluded</i>	N 12.78
10	July 27	N 23.06	S 24.40	N 13.46	S 22.23	S 20.19	N 1.35	S 4.83
Mag-N tests								
2	July 15	S 0.43	N 8.46	N 17.04	N 9.07	N 0.97	N 5.08	N 6.68
5	July 19	N 1.45	N 15.75	S 13.72	S 2.06	S 13.54	N 1.14	S 4.45
8	July 22	N 6.02	S 11.18	N 0.18	S 13.64	N 13.49	N 5.97	N 0.15
11	July 29	N 2.79	S 22.10	S 6.02	S 23.70	S 3.18	N 17.70	S 5.74
Mag-S tests								
3	July 16	N 23.55	N 4.75	<i>excluded</i>	N 16.08	N 11.35	N 15.06	N 14.15
6	July 20	N 6.81	N 18.47	S 3.56	S 9.96	N 16.69	N 5.46	N 5.66
9	July 25	N 15.32	S 18.47	<i>excluded</i>	S 12.19	N 23.29	S 0.28	N 1.52
12	August 1	X	X	N 13.84	<i>excluded</i>	<i>excluded</i>	S 8.89	N 2.49

^a Six newts (animals A–F) were tested in the sequence noted, with the bar magnet absent (Mag-0) or present at the north (Mag-N) or south (Mag-S) end of the tank corridor. Individual means are corrected to exclude positions when newts climbed on the tank wall and the mean is excluded altogether if the number of data points is less than 10. Climbing animals, i.e., those climbing on the tank walls 10 or more times during a given test, are indicated by italics. Mean values are given in centimeters, north (N) or south (S) of the center of the tank where each animal was released. Animals A and B died of unknown causes on August 1. From Phillips and Adler (1978).

salamander experiments, light was kept dim and diffuse to avoid influencing the response. Newts were exposed to three magnetic conditions: (1) the natural magnetic field, (2) magnetic north tests (Mag-N) in which a bar magnet was placed horizontally 5 cm beyond the north end of the tank, and (3) magnetic south tests (Mag-S) in which the magnet was placed an equal distance from the tank's south end. Newts were observed for a 90-min period under each condition, and their position, as well as whether they were on the floor or climbing the walls, was noted. From comparison of the mean floor positions of six animals (Table I), and of the positions on the wall of climbing animals relative to the placements of the bar magnet, the authors concluded that newts are capable of perceiving magnetic cues and prefer positions where the field strength and inclination approximate ambient values.

The apparent existence of magnetic sensitivity in newts and cave salamanders suggests that the capability may be present in salamanders generally (Phillips and Adler, 1978). The two salamanders studied belong to markedly different habitats, thus their magnetic sensitivity probably did not evolve simply in response to specific environmental constraints, such as cave dwelling. While the terrestrial cave salamanders probably perceive the magnetic field directly, the newts, like other aquatic vertebrates, could indirectly perceive the field through electric induction. The two species studied thus far, however, are phylogenetically related rather closely, and it is likely that they share a common type of magnetoreceptor, possibly involving magnetite.

At this time, the two papers discussed above comprise the published research on magnetic sensitivity in amphibians. Many questions still remain to be answered. Are other amphibians sensitive to magnetic fields? How is such sensitivity used in orientation? Is magnetic perception in amphibians magnetite-mediated, or is some other sensory mechanism responsible? Studies under the direction of Dr. J. B. Phillips and Dr. K. Adler are currently in progress to examine magnetoreception in amphibians in greater detail.

3. Reptiles

Most reptile movements are confined within small-scale natural boundaries, such as the shores of a pond for aquatic turtles, or to a few kilometers of desert or forest terrain for the terrestrial snakes, lizards, and turtles. Only the sea snakes, the saltwater crocodile, and the marine turtles travel across long distances.

Little is known about the migratory patterns and mechanisms of the sea snakes (Dunson, 1975) or the crocodile (Minton and Minton, 1973). Sea snakes are without well-defined musculature, and are not capable of vigorous or prolonged swimming. It appears that these snakes are almost planktonic and drift passively with the currents, sometimes over hundreds or thousands of kilometers when they are caught up in the major ocean gyres. The crocodiles may be more adept at charting a course than the snakes, but their movements are almost wholly unresearched. Of course, long-distance migration is not a prerequisite for magnetic sensitivity in reptiles. Nonmigratory snakes, alligators, crocodiles, lizards, and turtles may use a magnetic sense for short-range orientation, but studies have not yet been conducted to test this hypothesis.

Among the reptiles, the best-researched long-distance migrant is the green turtle, *Chelonia mydas*. Green turtles can accurately navigate in open ocean waters over distances up to a few thousands of kilometers. Tagged individuals from populations throughout the world have been shown to migrate from coastal feeding pastures to far-removed breeding and nesting grounds on remote island beaches, returning to the same nesting sites repeatedly (Carr and Carr, 1970; Pritchard, 1973; Balazs, 1976). Hirth (1971) has suggested that green turtles employ a multiplicity of cues and several senses, possibly including magnetoreception, for navigation. Magnetic cues available to the turtles include the geomagnetic field's intensity, polarity, and inclination. Sea turtles could also use magnetic anomalies such as the striped pattern on the ocean bottom that is a result of continuous seafloor spreading during epochs of normal and reversed magnetic field direction, and volcanic islands and seamounts that are sites of magnetic anomaly due to the high iron content of the basaltic lavas that formed them.

A small amount of pilot work with green turtles and another of the migratory sea turtles, the loggerhead *Caretta caretta*, has produced some results which could be supportive of a magnetic sensitivity in these species.

3.1. Magnetoreception in Sea Turtles

Baldwin (1972) compared the headings in the wild of four radio-equipped green turtles that had magnets attached to their plastrons with their headings when equipped with nonmagnetic brass bars. On clear days or in shallow water, no differences in headings before and after magnet placement were apparent. However, the heading of one turtle tested in deep water under overcast conditions suggested that where the bottom is not visible, sensing of the magnetic field could be important in turtle orientation. Further experiments with the remaining turtles were hampered by difficulties with weather and with the radio-tracking equipment, and to our knowledge these experiments have not been repeated.

We have conducted preliminary learning experiments to test for magnetoreception in sea turtles. All our experiments were carried out at the National Marine Fisheries Service Laboratory in Honolulu, Hawaii. We first tested the ability of two 1-year-old green turtles to discriminate between normal and altered magnetic field conditions (Perry, 1982). The turtles were tested for magnetic sensitivity using a discrete-trials/fix-interval conditioning technique (Woodward and Bitterman, 1974). Experiments were conducted in a 6-m-diameter, circular, nonmagnetic pool that was wrapped with a Helmholtz coil constructed

of 100 turns of magnet wire. A 1-A dc current through the coil added a vertical field of from 0.30 G at tank center to 0.50 G along the periphery to the Hawaiian magnetic field of about 0.35 G. The turtles were trained to repeatedly press a paddle presented at tank edge. At the end of 30-sec trial periods, they were rewarded with a small piece of fish. The paddle was manually lowered into the tank at the beginning of each trial by the experimenter, who was stationed behind a screen. A bar attached perpendicularly to the paddle handle enabled the experimenter to lower the paddle while remaining out of the turtle's view. A microswitch attached to the back of the paddle and connected to an automatic counter recorded the number of presses per trial.

For discrimination testing, the turtles were rewarded for pressing the paddle only during stimulus-present (S^+) trials. One turtle (turtle A) was rewarded for paddle presses in the altered magnetic field, the other (turtle B) for presses in the normal field. Each trial began after a random interval of 20–60 sec with simultaneous presentation of the paddle and the altered or normal magnetic field. For S^+ trials, the first paddle press after 30 sec had elapsed resulted in reward presentation and paddle removal. During stimulus-absent (S^-) trials, presses during the 30-sec period earned nothing and, as punishment, the paddle was not removed for another 20 sec, thus delaying the start of a new trial. Paddle presses during this punishment period resulted in subsequent 20-sec delays in paddle removal, up to 60 sec total. S^+ and S^- trials were presented in quasi-random order, with no more than three trials of one type in a row. Each day's session consisted of 20 S^+ and 20 S^- trials.

After the first 3 days of discrimination testing, turtle A's response rate during the S^+ condition was significantly greater than during S^- trials for 5 days (200 trials), using a *t*-test for paired comparisons ($t_4 = 6.4$, $p < 0.01$). In later trials, however, correct responding by turtle A did not exceed 50%, and the experiment was halted (Fig. 2a). Although there were no changes in the experimental setup for trials with turtle B, except to reverse the rewarded and unrewarded conditions, turtle B never showed the ability to discriminate between the two field conditions (Fig. 2b).

We also conducted learning experiments with an adult male loggerhead turtle of approximately 20–25 years of age. The experiments were conducted in one arm of a U-shaped concrete tank. A free operant conditioning format was used in which the subject was reinforced for pressing a paddle in the presence of a magnetic stimulus and was not reinforced for pressing in the absence of the stimulus. A buzzer (secondary reinforcer) followed by a fish (primary reinforcer) was delivered after every correct paddle press, i.e., a continuous reinforcement (CRF) schedule was employed. Presentation of magnetic stimuli and recording of data were fully automated; food was delivered by an experimenter blind to the stimulus condition. There were no adverse experimental contingencies for incorrect responding. S^+ and S^- trials were counterbalanced over groups of 30 trials for experiment I and of 12 trials for experiment II. It should be noted that the S^- condition was not equivalent to the normal Hawaiian magnetic intensity of about 0.35 G. Steel reinforcing bars in the walls of the tank distorted the inclination and reduced the intensity to 0.27 G at the experimental paddle.

In experiment I a small Helmholtz coil was placed perpendicularly to the N–S axis of the experimental tank. This generated a graded field which amplified the horizontal component of the ambient field and produced an overall intensity of 0.77 G at the point of response, the paddle. Dc current was used to reduce acoustic artifacts, as ac generates a 60-cycle hum.

As in the green turtle trials, the results from this experiment were somewhat ambiguous. Approximately 540, 30-sec trials were run over 6 days before performance stabilized at 50% correct response. Over the following 4 days of trials (90 trials a day), performance exceeded 50% on 3 of 4 days (range 48–54%). There was some indication of a deterioration in performance at the end of each session, perhaps due to reduced motivation. If the last

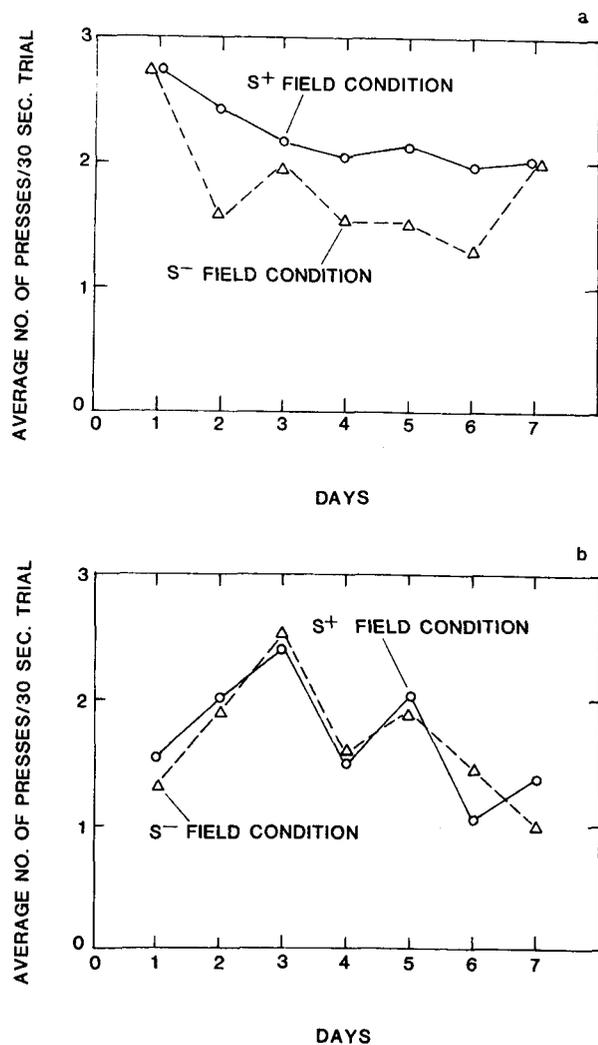


Figure 2. Daily averaged rate of paddle presses by turtle A (a) and turtle B (b) after the first 3 days of discrimination testing.

30 trials for each session are dropped from consideration, the subject exceeded 50% on all 4 days (range 51–60%). Data considered over counterbalanced groups of 30 trials (15 S⁺ and 15 S⁻ trials) indicated a significant difference using a *t*-test for paired comparisons ($t_7 = 2.67, p < 0.05$).

In experiment II the same free operant procedure was used, but the coil was suspended horizontally above the subject. A field was generated which added to the vertical component of the ambient field and produced an intensity of 0.58 G at the point of response. Because of the turtle's relative stability in the horizontal plane, we felt that a vertical field alteration would present the subject with a more stable stimulus as it moved within the experimental area. Performance over 266 discrimination trials, however, remained at ap-

proximately 50%. In other words, the turtle was responding equally in S^+ and S^- conditions, i.e., at chance levels.

Although there were differences in inclination and intensity between the two loggerhead experiments, and between the loggerhead and green turtle experiments, methodological deficiencies preclude any intelligent discussion of differences in our results based on these factors. The results of the experiments were equivocal from several standpoints:

1. The slow consummatory response of the loggerhead deprived it of response time during the S^+ period, possibly artificially depressing discriminative performance.
2. A free operant procedure, especially using a CRF, allows the subject to track reinforcement, i.e., the act of rewarding a response increases the probability of another response no matter what the state of the discrimination stimulus. In other words, a better than 50% performance might be attained solely by using reinforcement cues rather than magnetic cues.
3. The effect, when present at all, was small.

Due to differences in experimental design, items (1) and (2) were not problems in the green turtle study, but the effect was also small (54–58% correct response) during trials with turtle A, and was absent in turtle B's experiment. Under such circumstances, one has to be concerned with subtle alternative cues or chance effects.

If one keeps the results of these discrimination experiments in perspective, they can be considered promising pilot work. There are various procedural changes that can be employed in future experiments which will reduce the ambiguity of results.

1. There are a number of learning formats which can reduce or eliminate reinforcement cues. These include lean partial reinforcement schedules, testing on extinction trials, and the discrete-trials/fixed-interval method reported by Woodward and Bitterman (1974) and used in the green turtle experiments (see also Walker *et al.*, Chapter 20, this volume).

2. Punishment, e.g., time-out, or delays before the beginning of another trial, has been shown to be effective in reducing incorrect responses in discriminatory learning by green turtles (Manton *et al.*, 1972) and in discriminatory learning of magnetic cues by tuna (M. Walker, personal communication). Mild punishment procedures (delay of paddle removal) were used in the green turtle experiment, and might prove effective in training loggerheads and other turtles in magnetic discrimination formats.

3. The use of only one or two subjects and a single two-choice format leaves open to question the influence of nonexperimental cues, i.e., discriminative cues other than the magnetic field which may bias responding. Using single subject designs, the influence of alternative cues can be lessened by reversals of reinforcement contingencies. For example, initially reward responses during the presence of a magnetic field but not in its absence. After a criterion percentage correct is attained, reverse the contingencies, reward only responses in the absence of the magnetic field, but not in its presence. In this manner, cues that might accompany one magnetic condition but not the other are equated over the experiment. Another alternative is to increase the sample size and make reward contingencies differ for different groups, i.e., one group is only rewarded in the presence of a magnetic stimulus, the other is rewarded only in the absence of the magnetic stimulus.

4. Fixing the presentation of food at a given site and fully automating the feeding procedure can reduce potential experimenter cues as well as encourage a more time efficient consummatory response by the subject.

5. The use of more magnetically clean procedures can enhance the likelihood of a subject detecting a stimulus as well as giving the experimenter a clearer picture of exactly what stimulus characteristics are salient. For example, in the loggerhead experiments the steel reinforcing bars in the tank walls created a disorderly ambient field and distorted the gradient of the experimental field.

Additional issues in discrimination learning experiments using magnetic stimuli are discussed in Ossenkopp and Barbeito (1978) and in the chapter on cetaceans (Bauer et al.) in this volume.

We are presently conducting a study on the resting orientation of green turtles in response to altered magnetic field conditions. In these experiments, individual turtles are placed in a small, indoor tank that is enclosed in black plastic to eliminate light and visual distractions. Helmholtz coils outside the tank are supplied with power to alter the normal magnetic field. Turtles are kept in the tank overnight in complete darkness, except for a brief flash every 20 min to provide light for an overhead camera that records their positions. The turtles are being tested for orientation responses to shifts in both the horizontal and the vertical components of the magnetic field. Similar experiments conducted with eels (Tesch, 1974) demonstrated a clear orientation shift with changes in the horizontal field components. We hope that the results from these tests will be less ambiguous than those obtained in our learning experiments, and that they will aid us in designing future experiments to determine what aspects of the field might be relevant to migrational navigation in the sea turtles.

3.2. Isolation and Identification of Magnetite in Sea Turtles

During the course of our operant conditioning experiments, we conducted magnetometry studies to look for magnetic material in *Chelonia mydas*. Specimens of hatchling, juvenile, and adult green turtles were examined for magnetic remanence using SQUID magnetometers. To reduce the risk of contamination, the magnetometer enclosure was thoroughly cleaned, and only nonmagnetic glass and wood instruments were used in all handling and dissection. Whole hatchlings and samples of tissue from all age classes were rinsed with glass-distilled water, frozen in liquid nitrogen, and saturated with a 3000-G cobalt/samarium magnet. Saturation isothermal remanent magnetization (sIRM) was then measured in the SQUID. Background signal measurements were taken periodically for comparison with tissue signals, and to ensure that the magnetometer enclosure remained magnetically clean.

Magnetic remanence was found in the head region of all turtles examined (four hatchlings, three juveniles, and two adults; Table II). The greatest concentration of magnetic material occurred in the anterior portion of the dura mater, although it was also present diffusely throughout the facial muscle. sIRM in the adult turtle duras was 9×10^{-6} emu, about 50 times greater than background. Because of this high remanence, dura tissue was the primary material examined in further tests.

Remanence was also found in whole hatchlings. When these were further dissected, the highest readings were obtained from the stomach; they probably came from magnetic particles present in sand and dirt ingested by the turtles. Remanence associated with surface tissues, such as carapace scutes, could be the result of external contamination and was therefore not measured separately or examined in subsequent tests.

The sIRM measured in the turtle is within the range of that found in other vertebrate species (see Bauer et al.; Presti; Walker et al., Chapter 20, this volume). Adult turtles contain more magnetic material than juveniles, a phenomenon also observed in yellowfin tuna (Walker et al., Chapter 20, this volume), honeybees (Gould et al., 1978), and woodmice (J. Mather, personal communication).

Remanence in the dura was found from alternating-field demagnetization to be magnetically hard. The median unblocking field for net magnetic alignment in the dura was approximately 225 G, which indicates that single-domain magnetite crystals are present in the dura samples (Kirschvink and Lowenstam, 1979).

Table II. sIRM in Three Age Classes of Green Turtles

Sample	emu
Whole hatchlings	3.8×10^{-6}
	8.7×10^{-6}
	1.3×10^{-5}
Hatchling heads	2.3×10^{-5}
	1.4×10^{-6}
Hatchling necks	1.6×10^{-6}
	7.0×10^{-8}
Juvenile heads	8.3×10^{-8}
	1.1×10^{-6}
Juvenile flippers	3.0×10^{-6}
	9.5×10^{-6}
Front left	3.7×10^{-7}
Front right	2.8×10^{-7}
Rear left	9.7×10^{-7}
Rear right	6.3×10^{-7}
Adult brain	3.2×10^{-7}
Adult eyeball	6.0×10^{-7}
Adult facial muscle blocks	6.3×10^{-7}
1 × 1 × 1 cm	4.4×10^{-7}
1 × 1 × 2 cm	5.5×10^{-7}
1 × 2 × 3 cm	4.4×10^{-6}
1 × 3 × 3 cm	6.1×10^{-6}
Adult dura mater	
Posterior	1.0×10^{-6}
Posterior	1.5×10^{-6}
Anterior	9.0×10^{-6}
Anterior	9.2×10^{-6}
Empty sample holder	4.2×10^{-8} – 2.3×10^{-7}

In order to identify the source of magnetic remanence in turtle tissue, we extracted and analyzed magnetic particles from adults and juveniles (for methods used see Walker *et al.*, Chapter 5, this volume). Particle samples were examined via X-ray diffraction, electron microprobe analysis, EDAX line spectra analysis, and scanning electron microscopy.

The X-ray diffraction pattern produced by aggregated particles from turtle duras is that expected for magnetite (Fig. 3). The lattice spacing parameter estimate calculated from this pattern was 0.8375 ± 0.004 nm, which compares well to the reference parameter for magnetite of 0.8396 nm, indicating that we had extracted a very pure magnetite sample from the turtles. The number and relative intensities of rings in the pattern, specific for each crystal type, also closely match the reference values (Joint Committee on Powder Diffraction Studies, 1974).

Electron microprobe analysis revealed that the extracted particles were very rich in iron and contained no measurable titanium or chromium, as would be expected for magnetite isolated from rock. Unlike geologic magnetite, small amounts of manganese and calcium oxides were persistently associated with the crystals (Table III). Tissue residue often found associated with the magnetite particles after extraction (see Walker *et al.*, Chapter 5, this volume) may account for the presence of these oxides. Analysis by EDAX probe corroborated the microprobe data, exhibiting strong peaks only for iron, and showing

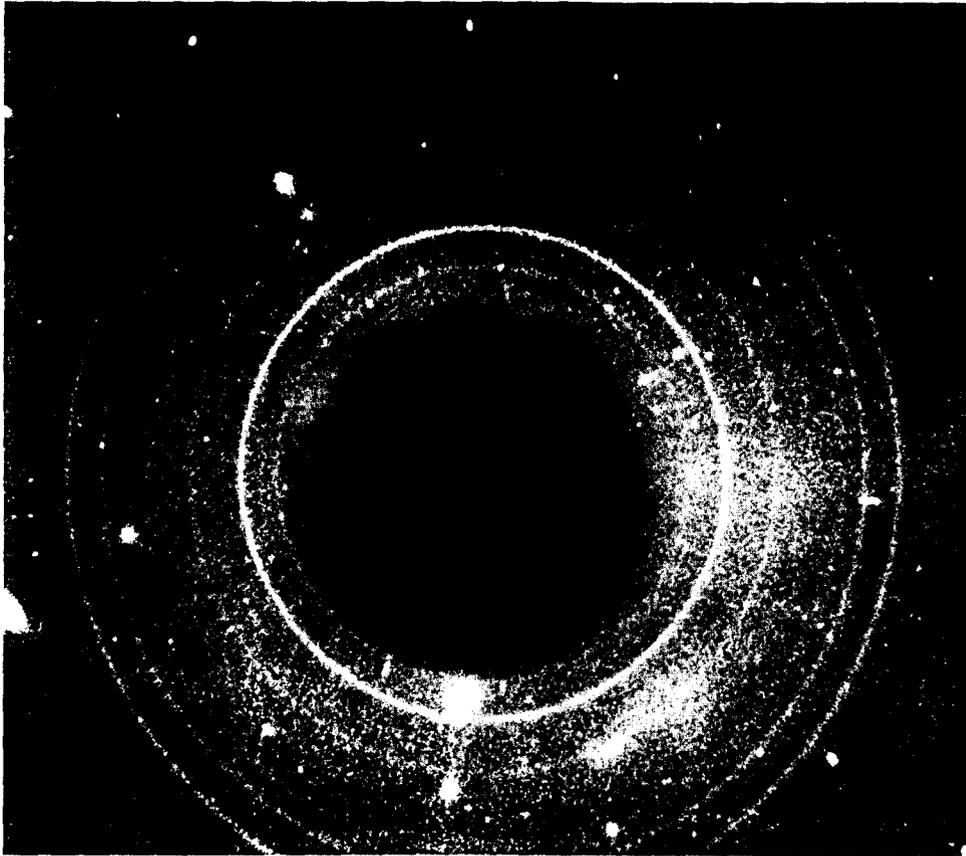


Figure 3. X-ray diffraction pattern of magnetite extracted from green turtle dura maters.

Table III. Electron Microprobe Analysis of Magnetite Particles Isolated from Green Turtles

Oxide	Weight % of sample	
	Magnetite standard (NMNH 11487)	Turtle
FeO	90.9	85.5 ± 1.7
TiO ₂	0.2	0.0 ± 0.0
Cr ₂ O ₃	0.2	0.0 ± 0.0
MnO	0.0	0.3 ± 0.0
CaO	—	0.3 ± 0.1
Total	91.3	86.1

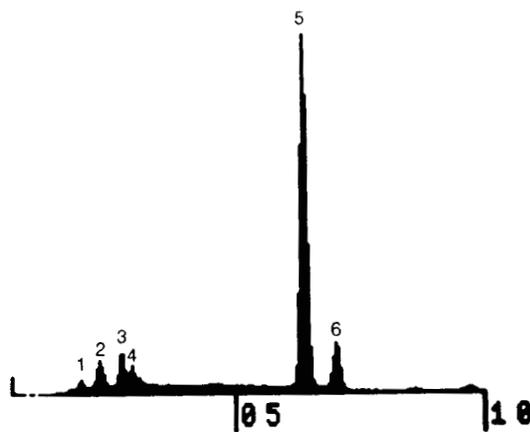


Figure 4. EDAX spectrum for magnetite sample from green turtle dura maters. Numbered peaks correspond to the following elements: 1, silica (from glass coverslip sample was placed on); 2, gold (from gold/paladium coating sprayed on sample to increase conductivity for scanning electron microscopy); 3, chlorine (from bleach used to digest dura membrane tissue); 4, paladium (from gold/paladium coating); 5 and 6, iron.

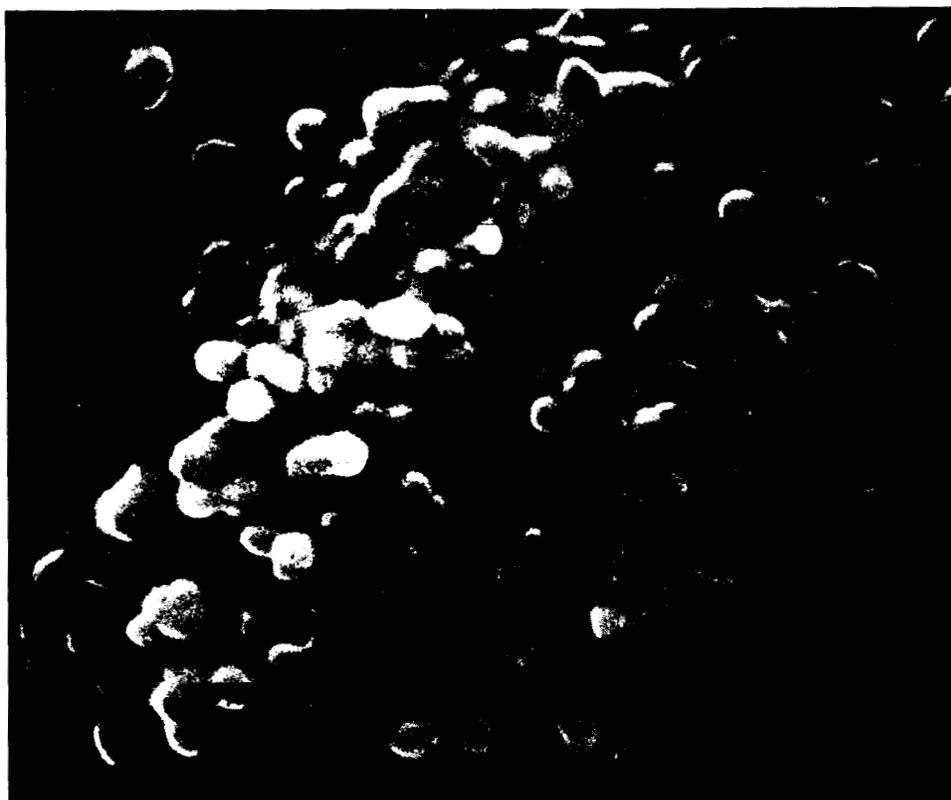


Figure 5. Scanning electron micrograph of colloidal crystal structure from green turtle dura mater sample.

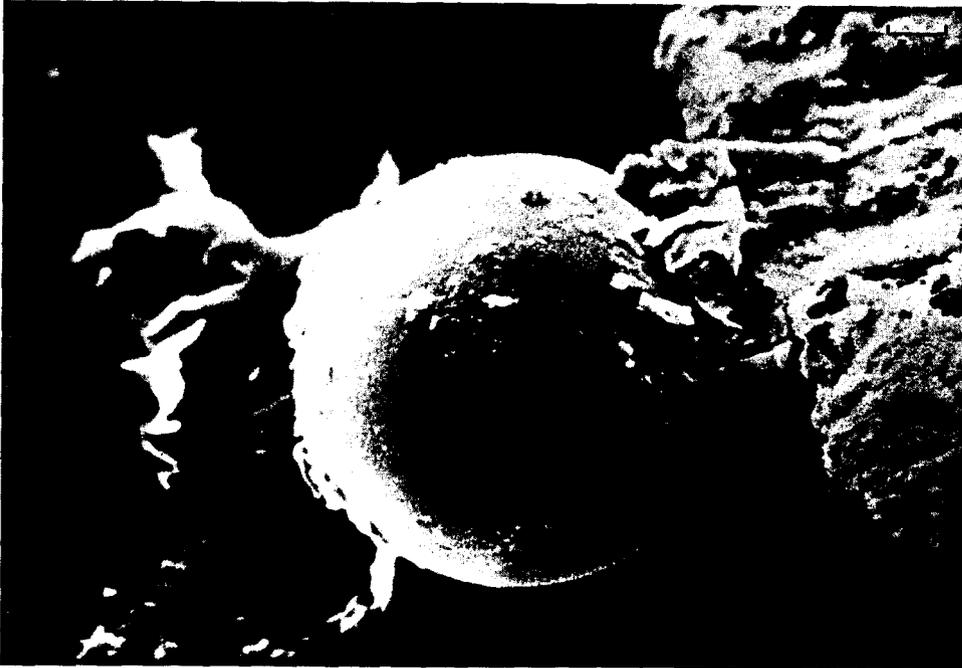


Figure 6. Scanning electron micrograph of sphere from green turtle dura mater sample.

no presence of other metals that would be expected as geologic contaminants, including nickel (Fig. 4).

Scanning electron microscopy revealed two types of crystal structure in dura samples: colloids (Fig. 5), and spheres (Fig. 6). The colloidal structures may comprise clusters of single-domain magnetite crystals. Although the apparent size of each nodule in the cluster is larger than that which would produce single-domain behavior in magnetite, each crystal may be surrounded by an organic coat. The spheres of magnetite, such as the one depicted in Fig. 6, have not previously been observed in organisms, although magnetite-containing cosmic spherules are common (Parkin *et al.*, 1977). It is possible that the turtle dura spheres are not of biological origin, but are contaminating cosmic spherules. We believe, however, that the spheres we observed are the result of biologic precipitation rather than cosmic contamination for several reasons. Our sample preparations were carried out in two different laboratories at different times, and as cosmic spherules are not a common laboratory contaminant it seems unlikely that they would appear in both sample sets. Cosmic spherules usually contain high proportions of magnesium and nickel, neither of which were present in our samples. Our strongest evidence for *in situ* information was that the spheres were not seen in control samples or in tissues other than dura. At this time the function of the large (10–50 μm) spheres is unknown. Dr. J. L. Kirschvink speculates that they may be involved in a system that detects geoelectric fields (Cromie, 1982).

The green turtle is a threatened species, and we therefore use only specimens that have died from natural causes in our magnetometry studies. As specimens become available, we hope to isolate more spheres from the duras and to study their properties in greater detail. We can then attempt to elucidate the role the spheres play, if any, in turtle magnetoreception.

4. Conclusion

The amphibians and reptiles form an evolutionary link between higher and lower vertebrates. Because of this phylogenetic position, the examination of magnetic perception and the sensory apparatus responsible for such perception is of particular interest in these classes. Evidence is accumulating for magnetoreception and magnetite biomineralization among fish, mammals, and birds. Preliminary work on magnetic susceptibility in salamanders and turtles, and the discovery of magnetite in turtles, is an important step toward understanding the evolutionary processes involved in magnetic sensitivity in all vertebrates. As demonstrated by this chapter, research on magnetoreception in the amphibians and reptiles has just begun. Additional studies are needed if the role of magnetite and magnetic sensitivity in vertebrates as a group is to be clearly understood.

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