References and Notes

1. J. Leiter, M. B. Shimkin, M. J. Shear, *J. Natl. Cancer Inst.* **3**, 155 (1942).
2. P. Kotin, H. L. Falk, P. Mader, M. Thomas, *Arch. Ind. Hyg. Occup. Med.* **9**, 153 (1954).
3. Committee on Biologic Effects of Atmospheric Pollutants, *Particulate Polycyclic Organic Matter* (National Academy of Sciences, Washington, D.C., 1972).
4. D. Hoffman and E. L. Wynder, in *Air Pollution*, A. C. Stern, Ed. (Academic Press, New York, ed. 3, 1977), vol. 2, pp. 361-455; R. C. Lao, R. S. Thomas, H. Oja, L. Dubois, *Anal. Chem.* **45**, 908 (1973); R. C. Pierce and M. Katz, *Environ. Sci. Technol.* **9**, 347 (1975); D. Grosjean, in *Ozone and Other Photochemical Oxidants* (National Academy of Sciences, Washington, D.C., 1977), pp. 45-125; W. Cautreels and K. Van Cauwenberghe, *Atmos. Environ.* **10**, 447 (1976); M. L. Lee, M. Novotny, K. D. Bartle, *Anal. Chem.* **48**, 1566 (1976).
5. W. C. Hueper, P. Kotin, E. C. Tabor, W. W. Payne, H. Falk, E. Sawicki, *Arch. Pathol.* **74**, 89 (1962).
6. S. S. Epstein, S. Josh, J. Andrea, N. Mantel, E. Sawicki, T. Stanley, E. C. Tabor, *Nature (London)* **212**, 1305 (1966); R. H. Rigdon and J. Neal, *Tex. Rep. Biol. Med.* **29**, 110 (1971); A. E. Freeman, P. J. Price, R. J. Bryan, R. J. Gordon, R. V. Gilden, G. J. Kelloff, R. J. Huebner, *Proc. Natl. Acad. Sci. U.S.A.* **68**, 445 (1971); U. Mohr, H. Reznik-Schuller, G. Reznik, G. Grimmer, J. Misfeld, *Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe B* **163**, 425 (1976); G. Grimmer, *IARC (Int. Agency Res. Cancer) Sci. Publ. 16* (1977), pp. 29-39.
7. J. N. Pitts, Jr., Second Annual Progress Report, NSF-RANN grant AEN73-02904 A02, September 1975, p. V-8.
8. _____, D. Grosjean, T. M. Mischke, V. F. Simmon, D. Poole, *Toxicol. Lett.* **1**, 65 (1977).
9. _____, paper presented at the 174th national meeting of the American Chemical Society, Chicago, 28-31 August 1977; in *Biological Effects of Environmental Pollutants*, S. D. Lee, Ed. (Ann Arbor Science, Ann Arbor, Mich., in press).
10. B. N. Ames, J. McCann, E. Yamasaki, *Mutat. Res.* **31**, 347 (1975).
11. J. N. Pitts, Jr., paper presented at the Royal Society Meeting on Pathways of Pollutants in the Atmosphere, London, 3-4 November 1977; *Philos. Trans. R. Soc. London*, in press; paper presented at the Conference on Chemical Carcinogens in the Environment: Emissions and Control, Pasadena, Calif., 6-7 March 1978.
12. _____, W. L. Belser, K. Van Cauwenberghe, D. Grosjean, J. Schmid, D. R. Fitz, G. B. Knudson, P. Hynds, paper presented at the Environmental Protection Agency symposium on Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, Williamsburg, Va., 21-23 February 1978.
13. J. N. Pitts, Jr., D. Grosjean, K. Van Cauwenberghe, W. L. Belser, paper presented at the 175th national meeting of the American Chemical Society, Anaheim, Calif., 12-17 March 1978.
14. Also relevant to the postulated importance of oxidative processes during atmospheric transport of PAH are several reports of the carcinogenic activity of polar fractions of organic particulates (*5, 15*), products of ozonized gasolines (*16*), and products of oxidation of aliphatic hydrocarbons (*17*), and of the toxicity of the photooxidation products of a commercial fuel oil (*18*).
15. S. S. Epstein, N. Mantel, T. W. Stanley, *Environ. Sci. Technol.* **2**, 132 (1968); E. L. Wynder and D. Hoffman, *J. Air Pollut. Control Assoc.* **15**, 155 (1965); R. J. Gordon, R. J. Bryan, J. S. Rhim, C. Demoise, R. G. Wolford, A. E. Freeman, R. J. Huebner, *Int. J. Cancer* **12**, 223 (1973); S. Asashina, J. Andrea, A. Carmel, E. Arnold, Y. Bishop, S. Joshi, D. Coffin, S. S. Epstein, *Cancer Res.* **32**, 2263 (1972).
16. P. Kotin, H. L. Falk, C. J. McCammon, *Cancer* **11**, 473 (1958).
17. P. Kotin, H. L. Falk, M. Thomas, *ibid.* **9**, 905 (1956).
18. R. A. Larson, L. L. Hunt, D. W. Blankenship, *Environ. Sci. Technol.* **11**, 492 (1977).
19. D. M. Jerina, R. E. Lehr, H. Yagi, O. Hernandez, P. M. Dansette, P. G. Wislocki, A. W. Wood, R. L. Chang, W. Levin, A. H. Conney, in *In Vitro Metabolic Activation in Mutagenesis Testing*, F. J. de Serres, J. R. Fouts, J. R. Bend, R. M. Philpot, Eds. (Elsevier, Amsterdam, 1976), pp. 159-177; O. G. Fahmy and M. J. Fahmy, *Cancer Res.* **33**, 302 (1977).
20. H. L. Falk, I. Markul, P. Kotin, *AMA Arch. Ind. Health* **13**, 13 (1956); B. D. Tebbens, J. F. Thomas, M. Mukai, *Am. Ind. Hyg. Assoc. J.* **27**, 415 (1966); B. D. Tebbens, M. Mukai, J. F. Thomas, *ibid.* **32**, 365 (1971); K. W. Boyer and H. A. Laitinen, *Environ. Sci. Technol.* **9**, 457 (1975).
21. R. S. Berry and P. A. Lehman, *Annu. Rev. Phys. Chem.* **22**, 47 (1971).
22. "PAH are chemically inert and hence are removed from the air only by rain or the slow sedimentation of the particulates," according to L. Fishbein, in *Chemical Mutagens*, A. Hollaender, Ed. (Plenum, New York, 1976), vol. 4, pp. 219-319; see p. 232.
23. No mutagenic activity was found in any of the control runs performed with blank filters.
24. A 0.5-ml portion of S9 mix contains 0.02 ml of liver S9 from Aroclor-induced rats. We recently stressed (*12*) the need for standardization of the widely used Ames test (number of cells per plate, plate volume, concentration of rat liver S9, and so on).
25. Authentic samples of the three purified quinones (BP-6,12-quinone, BP-1,3-quinone, and BP-3,6-quinone) were tested in our laboratory and were found to be nonmutagenic in the Ames assay system. Note that exposure of BP to pure air (control run, see Fig. 1) also yielded small amounts of BP-quinones.
26. M. J. S. Dewar, T. Mole, D. S. Urch, E. W. T. Wasford, *J. Chem. Soc. (London)* (1956), p. 3572.
27. Cochromatography of the two TLC bands with authentic samples further confirmed these assignments. The two isomers present in the orange TLC band were resolved by gas chromatography, using a glass capillary column. Relative concentrations of the two isomers were 1:1 in the mixture synthesized according to Dewar *et al.* (*26*) and 10:1 in the orange TLC band formed on exposure of BP to NO_2 (plus a trace of HNO_3).
28. The reduction in reversion frequency of TA100 may be explained by microsomal detoxification of the sample. The enzyme systems activated by Aroclor in rat liver have the general function of detoxifying the insulting compound or compounds. These reactions sometimes form mutagens from nonmutagenic chemicals, which is the basis for the metabolic activation in the Ames test. The same or related enzymes may also catalyze reactions that reduce the activity of direct chemical mutagens.
29. M. J. S. Dewar and T. Mole, *J. Chem. Soc. (London)* (1956), p. 1441; H. Hopff and H. R. Schweizer, *Helv. Chim. Acta* **42**, 2315 (1957).
30. Control runs were also performed at each location, replacing the downstream filters by blank filters. No activity was detected in these control runs, indicating that no mutagens were transferred from the upstream to the downstream filter and that no mutagens were formed by interaction of ambient gaseous pollutants with the blank filters during sampling.
31. Riverside is located about 100 km east of Los Angeles.
32. O. L. Chapman, D. C. Heckert, J. W. Reasoner, S. P. Thackaberry, *J. Am. Chem. Soc.* **88**, 5550 (1966).
33. We irradiated in air 9-nitroanthracene adsorbed on silica gel and found significant yields of 9,10-anthraquinone (unpublished work). This is consistent with the results obtained in solution by Chapman *et al.* (*32*) and supports our suggestion that nitro-PAH adsorbed on airborne particles may be further photooxidized to quinones in sunlight.
34. R. C. Pierce and M. Katz, *Environ. Sci. Technol.* **10**, 45 (1976).
35. Y. Wang, R. Talcott, R. Sawyer, S. Rappaport, E. Wei, paper presented at the Environmental Protection Agency symposium on Application of Short-Term Bioassays in the Fractionation and Analysis of Complex Environmental Mixtures, Williamsburg, Va., 21-23 February 1978.

36. Supported by National Science Foundation-Research Applied to National Needs grant ENV 73-02904 A03. We appreciate helpful discussions with O. Chapman. We also thank R. A. Graham and E. C. Tuazon, who performed the infrared analyses of our NO_2-air mixtures; G. W. Harris, who measured the yields of nitro-PAH by ultraviolet spectroscopy; and T. L. Gibson, who performed several extractions.

13 March 1978; revised 17 July 1978

Growth Lines in a Bivalve Mollusk: Subdaily Patterns and Dissolution of the Shell

Abstract. *Scanning electron micrographs of sections of the prismatic shell of the bivalve* Mercenaria mercenaria *reveal narrow subdaily growth striations. The width of these narrow lines, formed by concentrations of organic material, corresponds to the quantity of shell material that would be expected to dissolve during periods of anaerobic metabolism. The* pH *in the extrapallial fluid of the bivalve decreases when the valves are closed, and the amount of dissolution of shell is related to the duration of valve closure.*

The shells of numerous mollusks contain repeating microscopic structures that have been interpreted as records of periodic growth (*1*). In radial sections of bivalve shells these features appear as lines or striations parallel with growth surfaces (*2, 3*). The temporal frequency of striations has been analyzed in various species and correlated with seasonal growth rates, geochronometry, and behavior and, by inference, with physiological changes in the animals studied (*1-4*). Growth lines are visible in polished and etched shell sections as narrow bands in which calcium carbonate is absent or much lower in concentration than in adjacent areas (*5*).

Until quite recently, hypotheses concerning the origin of these lines were based on an assumption of periodic episodes of calcium carbonate deposition (*6*), although a few references were made to the possibility that dissolution of recently formed shell might play a part (*3, 6*). Last year, Lutz and Rhoads (*7*) published a theory of growth-line formation which held that organic striations are simply residues left behind as a result of dissolution of shell material during periods of anaerobiosis. To date, quantitative evidence confirming this mechanism has not appeared. In this report we present independent data in partial support of the hypothesis of Lutz and Rhoads, based on measurements of extrapallial fluid *p*H (*8*), published rates of shell dissolution during valve closure (*9*), thickness of subdaily growth lines, and duration of closure.

We installed glass microelectrodes, sealed by O rings from the surrounding seawater, through a drilled hole in the

0036-8075/78/1103-0519$00.50/0

shell and into the extrapallial fluid of specimens of the hard clam *Mercenaria mercenaria* Linné. By cementing each bivalve to a glass plate we were able to monitor movement of the upper valve with a photovoltaic device, and simultaneously record the output of the photocell and the electrode on a strip-chart recorder. A sample of such data, obtained with animals in a 14:10 light-dark cycle and continuously submerged, appears in Fig. 1. Analysis by fast Fourier transform (*10*) of the simultaneous curves substantiated the conclusions of earlier investigators (*9*): we found a close correlation between valve position and acidity of the extrapallial fluid (Fig. 2). Results of previous work (*11*) suggested that *M. mercenaria* and other bivalves display diurnal and tidal rhythmicities in shell opening behavior. Although these conclusions (*11*) have been strongly criticized on the basis of statistical treatment (*12*), a more recent study in which experimental conditions were similar to those reported here supported the idea of circadian locomotor rhythms in *M. mercenaria* (*4*). Our animals, however, showed rhythms of shell movement in which periods ranged from 5.4 to 12.8 hours in different individuals (Fig. 3). It is not known whether subtidal mollusks show similar behavior under field conditions, but such short periods may be important with regard to so-called subdaily striations observed in several bivalve species (*3*). We therefore examined the ultrastructure of the valves of experimental animals to determine whether a relationship existed between deposition of growth lines and shell movement.

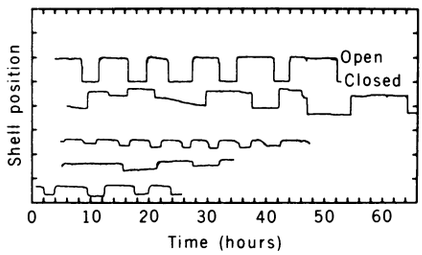


Fig. 1 (top left). Valve movement records for five specimens of *Mercenaria mercenaria*.

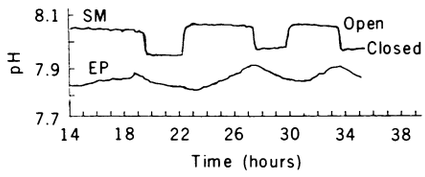


Fig. 2 (top right). Simultaneous record of valve movement (*SM*) and electrode potential in the extrapallial fluid (*EP*) of a specimen of *M. mercenaria*. Electrode potential increases slowly while the valves are open and decreases when they are closed.

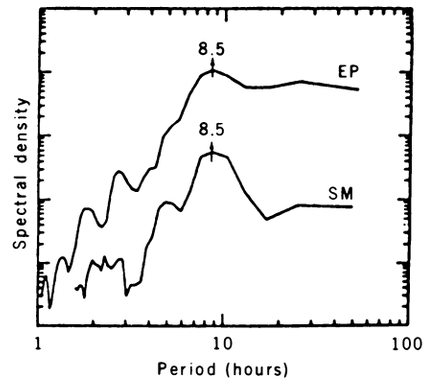
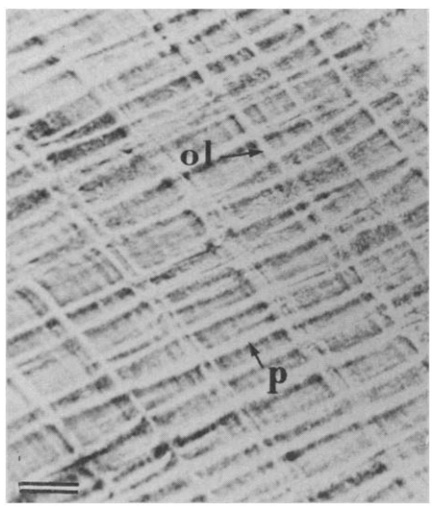


Fig. 3 (bottom right). Power spectrum, calculated by fast Fourier transform, of rhythms of shell movement (*SM*) and *p*H of the extrapallial fluid (*EP*) in a specimen of *M. mercenaria*. Major periods, in hours, are marked at peaks of spectral density.

Radial sections of *M. mercenaria* valves were polished and then etched with 0.5 percent HCl. As the organic matrix of the shell is less soluble than calcium carbonate in dilute acids, the effect of etching is to create a relief in areas with high concentrations of organic material. The sections were coated with gold and prismatic regions of shell were examined with a Cambridge Stereoscan microscope. It was not possible to clearly identify growth striations for what would have been the most recent 4 to 5 days of shell growth. This difficulty, possibly related to the lack of polymerization of the organic matrix near the growing margin of the shell (*13*), has been reported by others (*4*). We attribute such differences in appearance to the greater solubility of incompletely hardened proteins in our relatively concentrated etching solutions (*p*H 1.2) than in extrapallial fluids, in which the *p*H probably never falls below 6.5. However, away from the edge we obtained photographs showing well-defined series of single and double lines paralleling the growth surface (Fig. 4). At higher magnifications (Fig. 5) finer markings, which have been referred to as subdaily lines (*3*), were resolved, ranging in width between 0.45 and 0.9 μm. When sections were etched in 1*N* KOH the lines appeared as indentations between rows of crystallites. It is most likely, therefore, that the lines are composed of organic material with little or no calcium carbonate. If these are layers of hardened organic matrix remaining as residues after anaerobic dissolution, then recalcification does not occur after mineralized material is dissolved. We tested the hypothesis of dissolution by seeking a correlation between the amount of organic material in the layers and the period of time when the shell was exposed to a higher hydrogen ion concentration—that is, when the shell was closed.



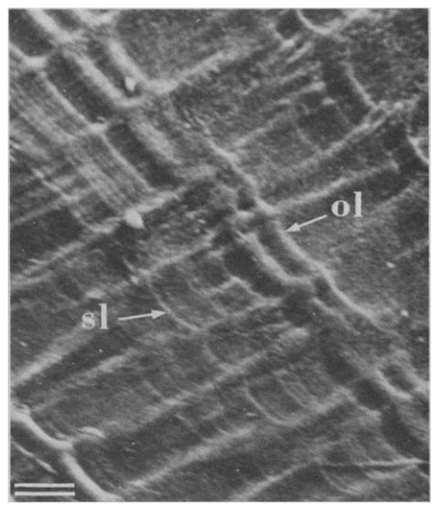


Fig. 4 (left). Scanning electron micrograph of radial section of mature prismatic shell, polished and etched with 0.5 percent HCl. Prism boundaries (*p*) and organic lines (*ol*) are clearly defined. Double lines parallel the growth surface. Scale bar, 25 μm.

Fig. 5 (right). Scanning electron micrograph of polished and etched prismatic shell. Subdaily lines (*sl*) can be distinguished from heavier tidal or diurnal organic lines (*ol*). Scale bar, 5 μm.

Crenshaw and Neff (*9*) showed that the calcium concentration in the extrapallial fluid of *M. mercenaria* continued to rise while the shell remained closed, at the same time that the hydrogen ion concentration was increasing. In addition, the increase in succinate, a product of anaerobic metabolism, in extrapallial fluid accounted for about 80 percent of the increase in dissolved calcium. Clearly the dissolution was not at chemical equilibri-

um, as the rate of addition of metabolic acids to the fluids was higher than the rate of neutralization by dissolved shell. Crenshaw and Neff (*9*) calculated that at the measured rates of calcium dissolution a 100-g animal (shell weight) would lose about 2 mg of shell per hour. We used animals with shell weights of 40 to 80 g and internal shell surface areas of 44 to 64 cm^2. Assuming the dissolution rate to be uniform over the entire internal surface, one can calculate the thickness of shell that would be removed for every hour the shell is closed

$$\frac{\text{shell loss per hour}}{\text{unit weight}} \times \frac{\text{total weight}}{\text{surface area}} \times \frac{1}{\text{density}} = \text{thickness lost per hour}$$

For example, one of our specimens with valves weighing 64 g and an internal surface area of 56 cm^2 would lose 7.8×10^{-2} μm/hour (using a density of 2.93 g/cm^3 for aragonite). Our experimental *M. mercenaria* kept their valves closed for 2.5 to 12 hours (Fig. 3), and at the calculated rate of shell dissolution the thickness removed would be 0.2 to 0.94 μm. These values are sufficiently close to the measured widths (0.45 to 0.9 μm) of subdaily lines to suggest a causative relationship between the two.

To account for subdaily striations, then, it is necessary only to envision continuous and simultaneous secretion of organic matrix and calcium carbonate during the aerobic shell-building part of the animal's growth cycle. In the anerobic period, increasing acid in the extrapallial fluid dissolves a portion of newly deposited shell. Some of the associated matrix may also dissolve, but at least part of it is sufficiently insoluble to resist attack by metabolic acids and remains behind as a residue, to be covered by a new layer of calcified material during the next cycle of aerobic deposition. Because the matrix at this point is hardened by polymerization of the protein, it maintains its structural integrity during and after decalcification. As a result, the width of residual matrix provides a record of the length of time that the shell was exposed to metabolic acids. This hypothesis, supporting that of Lutz and Rhoads (*7*), does not require alternate secretion of crystals and proteins, calcification inhibitors (*6*), or the existence of preformed matrix layers or compartments (*14*).

We recognize that this model of growth-line formation is at variance with current theory in the following respects. First, arrays of subdaily as well as tidal or diurnal markings are also found in the shell ultrastructure of intertidal mollusks. If, as is commonly accepted, these animals actively pump and deposit shell continuously during periods of inundation, then dissolution would not occur. Although we cannot safely assume that behavior in the laboratory is the same as that in the field, subdaily frequencies of shell opening have been reported elsewhere for experimental "nontidal" clams (*4*). Second, our hypothesis does not account for the thickness of presumed diurnal or tidal markings (*5*), which is as much as 3 μm in our experience and corresponds to 35 to 40 hours of shell dissolution according to our calculations (Figs. 3 and 4). Third, in certain areas of our sections, the accumulated thickness of subdaily markings found between two daily or tidal-diurnal lines is equivalent to more dissoluton than we would calculate for 24 hours of valve closure. This implies that one or more daily lines are missing from the depositional cycle. Evidence for missing growth lines in mollusks has been reported by others (*2*, *15*); however, Pannella (*5*), while admitting the possibility of gaps in the growth record, considers this exceptional.

Despite these questions, the correspondence between our calculated and observed subdaily line widths strongly suggests a connection between the appearance of these striations and rhythms of valve movement in *M. mercenaria*. The model of growth-line formation and shell dissolution (*7*) is thus quantitatively supported, at least for subdaily lines.

J. GORDON
M. R. CARRIKER
College of Marine Studies, University of Delaware, Newark 19711

References and Notes

1. G. R. Clark, *Science* **161**, 800 (1968); *Annu. Rev. Earth Planet. Sci.* **2**, 77 (1974); R. A. Lutz, *J. Mar. Biol. Assoc. U.K.* **56**, 723 (1976); M. W. House and G. E. Farrow, *Nature (London)* **219**, 1384 (1968).
2. G. E. Farrow, *Palaeontology* **14**, 571 (1971).
3. G. Pannella and C. MacClintock, *J. Paleontol.* **42**, 64 (1968); D. C. Rhoads and G. Pannella, *Lethaia* **3**, 143 (1970).
4. I. Thompson, in *Growth Rhythms and the History of the Earth's Rotation*, G. D. Rosenberg and S. K. Runcorn, Eds. (Wiley, London, 1975), p. 149.
5. G. Pannella, in *ibid.*, p. 253.
6. C. Gregoire, *Biol. Rev.* **42**, 653 (1967); K. M. Wilbur, in *Chemical Zoology*, vol. 7, *Mollusca*, M. Florkin and B. T. Scheer, Eds. (Academic Press, New York, 1972), p. 103.
7. R. A. Lutz and D. C. Rhoads, *Science* **198**, 1222 (1977).
8. Because we did not use calibration buffers of suitable ionic strength, we actually measured electrode potential rather than hydrogen ion concentration. Our graphs were converted to *p*H units for convenience.
9. M. A. Crenshaw and J. M. Neff, *Am. Zool.* **9**, 881 (1969).
10. J. W. Cooley and J. W. Tukey, *Math. Comput.* **19**, 290 (1965).
11. M. F. Bennett, *Biol. Bull.* **107**, 174 (1954); F. A. Brown, Jr., *Am. J. Physiol.* **178**, 510 (1954).
12. J. T. Enright, *J. Theor. Biol.* **8**, 426 (1965).
13. J. Gordon, thesis, University of Delaware (1978).
14. G. Bevelander and H. Nakahara, *Calcif. Tissue Res.* **3**, 84 (1969); D. F. Travis, C. J. Francois, C. L. Bonar, M. J. Glimcher, *J. Ultrastruct. Res.* **18**, 519 (1967).
15. G. E. Farrow, *Palaeontology* **15**, 61 (1972).
16. Supported by NSF grant BMS75-04842. Contribution No. 119 of the College of Marine Studies, University of Delaware.

24 March 1978; revised 11 July 1978

Radio Tracking of a Fin Whale (*Balaenoptera physalus*)

Abstract. *A tagged whale of the genus* Balaenoptera *was intermittently tracked by radio for 27.8 hours over a distance of about 145 kilometers. Data on breathing and movement show that during that time the whale took 58 breaths in 130 minutes and traveled 20 kilometers at more than 9 kilometers per hour. Precise measurements of such parameters and of other features of the life history of great whales, which travel long distances over the high seas, often in groups, are now possible through radio tagging.*

For many years scientists have recognized the need to mark whales in order to acquire basic biological data on such subjects as population identity, home range, migration, and behavior. The *Discovery* mark has been used for more than four decades, but its usefulness for these purposes is limited (*1*). An alternative is a visually detectable "streamer mark," which has the advantage that living whales may be followed, but this technique can be used only in the daytime in fair weather and requires the observer to be near the whale (*2*). Radio tags do not have these limitations.

We report here the remote implantation of a radio tag in a fin whale and subsequent tracking of the whale for 27 hours and 45 minutes. Our approach evolved from two earlier efforts. Schevill and Watkins (*3*) developed an implantable tag for right whales, *Eubalaena glacialis*, and Martin *et al.* (*4*) improved the response time of an automatic direction finder (ADF) for use in tracking porpoises. Subsequently, others have tracked whales by first capturing them and then attaching transmitters (*5*).

Whales on the high seas are among the most difficult animals to study. We chose

0036-8075/78/1103-0521$00.50/0