tracks (Fig. 1) show that the character of the magnetic anomalies is generally smoother and of longer wavelength for the approximately east-west trending tracks than for the north-south trending tracks.

The Mesozoic sequence of magnetic anomalies from M9 (~ 121 million years old) through M25 (~ 153 million years old) are symmetric about a ridge formed at the time of anomaly M9. These segments appear to be offset left-laterally from east to west and to be normal to the Davie fracture zone, but more magnetic data is required to confirm this and to determine if oblique sea-floor spreading occurred. The Jurassic magnetic quiet zone is observed on both sides of the ridge axes landward of magnetic anomaly M25. We identified a large amplitude magnetic anomaly on both landward sides of the Jurassic quiet zone. This anomaly may represent the ocean-continent boundary, as has been proposed for similar magnetic anomalies bordering other passive continental margins [for example, the magnetic anomaly off eastern North America (*18*) or anomaly G bordering the South Atlantic margin (*19*)]. The magnetic anomalies are disturbed in places by seamounts and islands in the southern part of the basin. Some of the islands have experienced volcanism since the Miocene (*20*).

The magnetic data point to the motion of Madagascar relative to Africa being from the north, with the Africa-Madagascar separation beginning during the time of the Jurassic quiet zone (~ 165 million years ago) and ending at a time of formation of anomaly M9 (~ 121 million years ago). The Africa-Madagascar separation thus began at about the same time as the breakup of Gondwanaland and the separation of North America from Africa. We used the Mesozoic time scales (*21*) for dating these anomalies, and derived half-spreading rates of 1.7 to 1.8 cm year^{-1}. We note that the results of recent deep sea drilling in the North Atlantic on the older parts of the Mesozoic sequence may reduce the age assigned to magnetic anomaly M25 and the Jurassic quiet zone (*22*) and hence increase the spreading rates.

Deep Sea Drilling Project (DSDP) site 241 (*23*) is located on the lower continental rise off northeastern Kenya and southeastern Somalia in the Jurassic quiet zone and just landward of magnetic anomaly M25. A multichannel seismic section from our recent *Vema* cruise across this site (*24*) is shown in Fig. 3.

Relative motion between Madagascar and Africa ceased ~ 121 million years ago, about 25 million years before creation of the purple reflector [Early Senonian (Fig. 3), corresponding to the age of the oldest sediment recovered during drilling]. Schlich *et al.* (*25*) and Simpson *et al.* (*23*) conclude, primarily from the drilling results, that the continental margin off northeastern Kenya and southeastern Somalia has been evolving passively for at least the last 90 million years and argue that it is unlikely that Madagascar occupied a position adjacent to that part of Africa during those 90 million years. Our marine magnetics data and seismic analysis are in good agreement with these drilling results (*23, 25*).

PHILIP D. RABINOWITZ
Department of Oceanography, Texas A & M University, College Station 77843, and *Lamont-Doherty Geological Observatory, Columbia University, Palisades, New York 10964*
MILLARD F. COFFIN
Lamont-Doherty Geological Observatory, Columbia University
DAVID FALVEY*
University of Sydney, New South Wales 2006, Australia

References and Notes

1. D. P. McKenzie and J. G. Sclater, *Geophys. J. R. Astron. Soc.* **25**, 437 (1971).
2. I. O. Norton and J. G. Sclater, *J. Geophys. Res.* **84**, 6803 (1979).
3. A. L. DuToit, *Our Wandering Continents* (Oliver & Boyd, Edinburgh, 1937).
4. A. G. Smith and A. Hallam, *Nature (London)* **225**, 139 (1970).
5. J. R. Heirtzler and R. H. Burroughs, *Science* **174**, 488 (1971); J. Segoufin and P. Patriat, *C. R. Acad. Sci.* **291**, 85 (1980).
6. P. D. Rabinowitz, *J. Geophys. Res.* **76**, 7107 (1971).
7. R. A. Scrutton, *Earth Planet. Sci. Lett.* **39**, 84 (1978); _____, W. B. Heptonstall, J. H. Peacock, *Mar. Geol.* **43**, 1 (1981).
8. E. T. Bunce and P. Molnar, *J. Geophys. Res.* **82**, 5305 (1977).
9. M. W. McElhinny, *Nature (London)* **228**, 977 (1970); _____ and B. J. J. Embleton, *Earth Planet. Sci. Lett.* **31**, 101 (1976); _____, L. Daly, J. P. Pozzi, *Geology* **4**, 455 (1976); B. J. J. Embleton, J. J. Veevers, B. D. Johnson, C. M. Powell, *Tectonophysics* **61**, 381 (1980); C. M. Powell, B. D. Johnson, J. J. Veevers, *ibid.* **63**, 13 (1980); B. J. J. Embleton and M. W. McElhinny, *Earth Planet. Sci. Lett.* **58**, 141 (1982).
10. R. T. Cannon, W. M. N. S. Siambi, F. M. Karanja, *Earth Planet. Sci. Lett.* **52**, 419 (1981).
11. P. D. Rabinowitz, M. F. Coffin, D. Falvey, *Science* **215**, 663 (1982).
12. A. G. Green, *Nature (London)* **236**, 19 (1972).
13. G. Flores, *Trans. Geol. Soc. S. Afr.* **73**, 1 (1970).
14. J. B. Wright and P. McCurry, *Earth Planet. Sci. Lett.* **8**, 267 (1970); E. J. Barron, C. G. A. Harrison, W. W. Hay, *Eos* **59**, 436 (1978); E. J. Barron and C. G. A. Harrison, in *Mechanisms of Continental Drift and Plate Tectonics*, P. A. Davies and S. K. Runcorn, Eds. (Academic Press, New York, 1980), p. 89; _____, J. L. Sloan, W. W. Hay, *Eclogae Geol. Helv.* **74**, 443 (1981).
15. P. E. Kent, *Nature (London)* **238**, 147 (1972); in *Implications of Continental Drift to the Earth Sciences*, D. H. Tarling and S. K. Runcorn, Eds. (Academic Press, New York, 1973), pp. 873 and 949; M. F. J. Flower and D. F. Strong, *Earth Planet. Sci. Lett.* **7**, 47 (1969); D. H. Tarling, *Nature (London)* **229**, 17 (1971); *ibid.* **238**, 92 (1972); _____ and P. E. Kent, *ibid.* **261**, 304 (1976); B. W. Darracott, *Earth Planet. Sci. Lett.* **24**, 282 (1974); F. Förster, *Palaeogeog. Palaeoclimatol. Palaeoecol.* **17**, 267 (1975).
16. T. J. Francis, D. Davies, M. N. Hill, *Phil. Trans. R. Soc. London Ser. A* **259**, 240 (1966).
17. J. Segoufin, *C. R. Acad. Sci. Ser. D* **287**, 109 (1978); E. S. W. Simpson, J. G. Sclater, B. Parsons, I. Norton, L. Meinke, *Earth Planet. Sci. Lett.* **43**, 260 (1979).
18. C. L. Drake, J. I. Ewing, H. Stockard, *Can. J. Earth Sci.* **5**, 993 (1968); K. O. Emery, E. Uchupi, J. D. Phillips, C. O. Bowin, E. T. Bunce, S. T. Knott, *Bull. Am. Assoc. Petr. Geol.* **54**, 44 (1970); P. D. Rabinowitz, in *The Geology of Continental Margins*, C. A. Burk and C. L. Drake, Eds. (Springer-Verlag, New York, 1974), p. 67; K. D. Klitgord and J. C. Behrendt, U.S. Geological Survey Miscellaneous Field Studies Map MF 913 (1977).
19. P. D. Rabinowitz and J. LaBrecque, *J. Geophys. Res.* **84**, 5973 (1973).
20. T. L. Vallier, *Init. Rep. Deep Sea Drill. Proj.* **25**, 515 (1974).
21. R. L. Larson and T. W. C. Hilde, *J. Geophys. Res.* **80**, 2586 (1975); S. C. Cande, R. L. Larson, J. L. LaBrecque, *Earth Planet. Sci. Lett.* **41**, 434 (1978).
22. F. Gradstein *et al.*, *Init. Rep. Deep Sea Drill. Proj.*, in press; J. E. T. Channell, J. G. Ogg, W. Lowrie, in preparation.
23. E. S. W. Simpson *et al.*, *Init. Rep. Deep Sea Drill. Proj.* **25**, 884 (1974).
24. M. F. Coffin and P. D. Rabinowitz, in *1982 Proc. Offshore Technol. Conf.* **2**, 421 (1982).
25. R. Schlich, E. S. W. Simpson, T. L. Vallier, *Init. Rep. Deep Sea Drill. Proj.* **25**, 743 (1974).
26. We thank the officers, crew, and scientists on the R.V. *Vema* for assistance in gathering the data and S. Cande and J. LaBrecque for critically reviewing the manuscript. Supported by grant OCE 79-19389. This is Lamont-Doherty Geological Observatory contribution 3447.
* Present address: Division of Mineral Geosciences and Petroleum Geology, Bureau of Mineral Resources, Canberra 2601, Australia.

9 August 1982; revised 1 November 1982

Fragile Sites in Chromosomes: Possible Model for the Study of Spontaneous Chromosome Breakage

Abstract. *The tissue culture condition that is required for the type of chromosome breakage seen at most fragile sites, namely, the absence of folic acid and thymidine in the medium, greatly enhanced micronucleus formation in proliferating lymphocyte cultures from normal individuals. This suggests that chromosome breakage at fragile sites and the apparently spontaneous damage that gives rise to micronuclei are controlled by the same mechanism.*

Fragile sites are heritable points on human chromosomes. Expressed as nonstaining gaps during metaphase, they are places where the chromosomes are very susceptible to breakage (*1*). The best evidence for this phenomenon is the formation of multiradial figures at metaphase, which arise from breakage and malsegregation of the chromosome fragment distal to the fragile site (*2*). Fragile

sites can be detected in preparations of lymphocyte chromosomes if the lymphocytes are grown in a tissue culture medium that is deficient in folic acid and thymidine (*1*).

Chromosome breakage in proliferating cells gives rise to micronuclei (*3*). The finding that micronuclei may appear more often in individuals with fragile sites than in individuals without them suggests that chromosome fragments resulting from breakage at such sites are eliminated by micronucleus formation (*4*). We report that, in lymphocytes from normal individuals, more micronuclei are formed when the cells are grown in medium lacking folic acid and thymidine than when they are grown in medium containing either of these compounds.

Blood from healthy volunteers was used to establish lymphocyte cultures. Each culture contained blood (0.2 ml), phytohemagglutinin (0.1 ml), penicillin (100 U/ml), and 5 ml of minimum essential medium free of folic acid and thymidine and supplemented with 5 percent fetal bovine serum (*1*). Replicate cultures were grown in this medium or in the same medium containing folic acid (5 mg/liter) or thymidine (10 mg/liter). After 5 days micronucleus frequency reached a maximum (*4*) and the cells were harvested by standard techniques. Slides were randomized and coded and micronucleus frequency was determined by examining 500 interphase nuclei per culture. Only nuclei that showed evidence of blastic transformation were counted.

The average frequency of micronuclei in lymphocytes grown in medium without folic acid and thymidine was 4.4 percent, compared to 0.4 percent in medium with folic acid or thymidine (Table 1). Micronucleus frequency varied in accordance with the concentration of folic acid and thymidine in the culture medium (Fig. 1). The dose-response relation is very similar to that seen for the expression of fragile sites under identical culture conditions (*1*).

Table 1. Frequency of occurrence of micronuclei in cultured lymphocytes from normal volunteers. The lymphocytes were cultured for 5 days in minimum essential medium containing folic acid (FA; 5 mg/liter) or thymidine (T; 10 mg/liter), or neither. Values are percentages.

Donor	Age	Sex	FA	T	Neither
1	12	F	1.7	0.9	6.6
2	23	M	0.2	0.6	6.5
3	32	F	0.4	0.0	4.4
4	40	F	0.0	0.0	1.0
5	18	F	0.2	0.6	3.6
6	22	F	0.0	0.4	3.4
7	55	F	0.4	0.2	2.9
8	37	F	0.6	0.4	5.8
9	35	M	0.2	0.6	5.0
Mean			0.4	0.4	4.4

The influence of culture medium composition on spontaneous (*5*) and induced (*6*) chromosome breakage has been documented but has not generally been recognized to be of the magnitude reported here. An increase in the frequency of random and nonrandom gaps and breaks has been reported in chromosomes of lymphocytes cultured under conditions suitable for fragile site expression (*7–9*). Some of the more common nonrandom damage includes gaps and breaks at 1qter, 2q23, 3p14, 6q26, 9q13, and 13qter. These regions can mimic true fragile sites but have not been shown to be heritable or to give rise to the triradial figures that characterize fragile sites, and they are usually seen in only a small portion of chromosomes in metaphase (*9*). Investigators of chromosomal aberrations or micronucleus formation will need to take the concentration of folic acid and thymidine in the culture medium into account.

The suppression of chromosome damage in lymphocytes cultured under the same conditions that suppress the expression of fragile sites suggests that a similar mechanism underlies both the chromosome damage resulting from fragile sites and the apparently spontaneous damage exhibited by normal individuals. Expression of fragile sites probably depends on a reduced amount of thymidine monophosphate during the late stages of DNA synthesis. This can be achieved by reducing the concentration of folic acid and thymidine in the culture medium to virtually zero (*1*); by adding methotrexate, an inhibitor of folate metabolism, to folic acid–containing medium (*10*); or by adding fluorodeoxyuridine, an inhibitor of thymidylate synthetase, which converts uridine monophosphate to thymidine monophosphate (*11, 12*). This inhibition can be overcome by adding excess thymidine but not excess folic acid (*11*). Perhaps there is a general class of chromosome breakage, of which the heritable fragile sites are special cases, that results from a deficiency of DNA precursor substances. Further studies of the mechanism by which heritable fragile sites are expressed may lead to a wider understanding of the mechanisms of spontaneous chromosome damage.

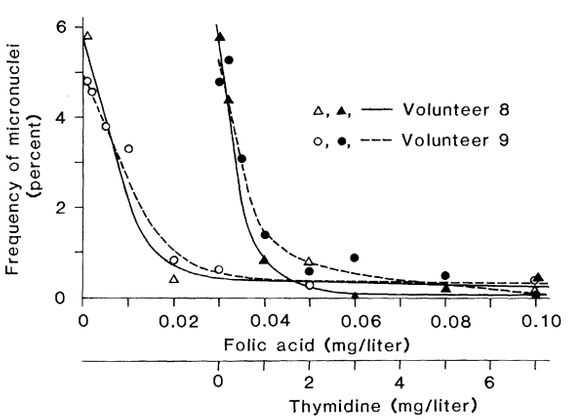

Fig. 1. Relation between micronucleus frequency in 5-day-old lymphocyte cultures and concentration of folic acid and thymidine in the medium for volunteers 8 and 9.

PETER B. JACKY
Cytogenetics Unit, Department of Histopathology, Adelaide Children's Hospital, North Adelaide, South Australia 5006, Australia
BERND BEEK
Institut für Genetik, Freie Universität Berlin, 1000 Berlin 33, Federal Republic of Germany, and *Cytogenetics Unit, Department of Histopathology, Adelaide Children's Hospital*
GRANT R. SUTHERLAND
Cytogenetics Unit, Department of Histopathology, Adelaide Children's Hospital

References and Notes

1. G. R. Sutherland, *Am. J. Hum. Genet.* **31**, 125 (1979).
2. ———, *Int. Rev. Cytol.* **81**, 107 (1983).
3. J. A. Heddle, R. D. Benz, P. J. Countryman, in *Mutagen-Induced Chromosome Damage in Man*, H. J. Evans and D. C. Lloyd, Eds. (Edinburgh Univ. Press, Edinburgh, 1978), pp. 191–200.
4. B. Beek, P. B. Jacky, G. R. Sutherland, *Ann. Genet.*, in press.
5. G. Obe, B. Beek, R. Slacik-Erben, *Eur. Soc. Toxicol. Int. Congr. Ser. No. 376* (1976), pp. 118–126.
6. B. Beek and G. Obe, *Experientia* **30**, 1006 (1974).
7. M. Jennings, J. G. Hall, H. Hoehn, *Am. J. Med. Genet.* 7, 417 (1980).
8. R. H. Martin, C. C. Lin, B. J. Mathies, R. B. Lowry, *ibid.*, p. 433.
9. M. A. Leversha, G. C. Webb, S. M. Pavey, *Lancet* **1981-I**, 49 (1981).
10. C. Fonatsch, *Hum. Genet.* **59**, 186 (1981).
11. T. W. Glover, *Am. J. Hum. Genet.* **33**, 234 (1981).
12. N. Tommerup, H. Poulsen, K. Brøndum-Nielsen, *J. Med. Genet.* **18**, 374 (1981).

29 September 1982