precipitate were visible, interspersed within a more particulate matrix. Possibly the particles represent aggregates of precipitate; the brush-like extrusions in the lightly loaded cell (Fig. 3A) apparently occur in discrete clumps, presumably corresponding to loci of phosphatase activity.

Whether the example of biocatalytically mediated crystal growth used by this *Citrobacter* sp. has evolved as a generalized mechanism of defense against metal toxicity by exocellular compartmentalization remains to be confirmed. Similarly, it is not known what role, if any, the architecture or integrity of the outer wall layers plays, or, indeed, whether the phosphatase itself is located in juxtaposition to the precipitate or distanced from it, within the periplasmic space.

REFERENCES AND NOTES

1. R. M. Aickin, A. C. R. Dean, A. K. Cheetham, A. J. Skarnulis, *Microbios Lett.* **9**, 7 (1979).
2. L. E. Macaskie, A. C. R. Dean, A. K. Cheetham, R. J. B. Jakeman, A. J. Skarnulis, *J. Gen. Microbiol.* **133**, 539 (1987).
3. L. E. Macaskie and A. C. R. Dean, *Adv. Biotechnol. Processes* 12, 159 (1989).
4. L. E. Macaskie, *J. Chem. Technol. Biotechnol.* **49**, 357 (1990).
5. ———, *Crit. Rev. Biotechnol.* 11, 41 (1991).
6. L. Strachan, L. E. Macaskie, B. C. Jeong, in *Proceedings of the 201st American Chemical Society Meeting* (American Chemical Society, Washington, DC, 1991), vol. 31, p. 128.
7. E. J. Plummer and L. E. Macaskie, *Bull. Environ. Contam. Toxicol.* **44**, 173 (1990).
8. L. E. Macaskie, J. D. Blackmore, R. M. Empson, *FEMS Microbiol. Lett.* **55**, 157 (1988).
9. G. Cliff and G. W. Lorimer, *J. Microsc. (Oxford)* 103, 203 (1975).
10. F. Weigel and G. Hoffman, *J. Less Common Met.* **44**, 99 (1976).
11. H. W. Dunn, *Oak Ridge Natl. Lab. Publ. 2092* (1956).
12. C. Greaves, A. K. Cheetham, B. E. F. Fender, *Inorg. Chem.* 12, 3003 (1973).
13. A. K. Cheetham, N. J. Clayden, C. M. Dobson, R. J. B. Jakeman, *J. Chem. Soc. Chem. Commun.* **1986**, 195 (1986).
14. J. Herzfeld and A. C. Berger, *J. Chem. Phys.* **73**, 6021 (1980).
15. W. O. Rothwell, J. S. Waugh, J. P. Yesinowski, *J. Am. Chem. Soc.* **102**, 2637 (1980).
16. A. R. Grimmer and U. Haubenreisser, *Chem. Phys. Lett.* **99**, 487 (1983).
17. T. M. Duncan and D. C. Douglass, *Chem. Phys.* **87**, 339 (1984).
18. B. Morosin, *Acta Crystallogr. Sect. B* **34**, 3732 (1978).
19. A. T. Howe and M. G. Shilton, *J. Solid State Chem.* **34**, 149 (1980).
20. *Citrobacter* sp. was pregrown to the midlogarithmic phase in minimal medium containing glycerol and glycerol 2-phosphate as the respective C and P sources (*8*). Cell harvests were resuspended in 2 mM citrate buffer (pH 6.9) supplemented with glycerol 2-phosphate (to 5 mM) and uranyl nitrate (to 1 mM) for 12 hours, to a final loading of U of about 250% of the cellular dry weight (*8*). In the examination of samples we used a Jeol JEM-2000 FX electron microscope with an accelerating voltage of 200 keV (*1*, *2*).
21. Financial support was provided by BTP Ltd., in association with Pembroke College, University of Oxford (to L.E.M.) and by the Science and Engineering Research Council (to A.K.C. and C.P.G.).

10 March 1992; accepted 29 May 1992

Increasing Rates of Atmospheric Mercury Deposition in Midcontinental North America

Edward B. Swain,* Daniel R. Engstrom, Mark E. Brigham,†
Thomas A. Henning,‡ Patrick L. Brezonik

Mercury contamination of remote lakes has been attributed to increasing deposition of atmospheric mercury, yet historic deposition rates and inputs from terrestrial sources are essentially unknown. Sediments of seven headwater lakes in Minnesota and Wisconsin were used to reconstruct regional modern and preindustrial deposition rates of mercury. Whole-basin mercury fluxes, determined from lake-wide arrays of dated cores, indicate that the annual deposition of atmospheric mercury has increased from 3.7 to 12.5 micrograms per square meter since 1850 and that 25 percent of atmospheric mercury deposition to the terrestrial catchment is exported to the lake. The deposition increase is similar among sites, implying regional or global sources for the mercury entering these lakes.

Mercury (Hg) contamination has been documented in many lakes in remote regions of Canada, Sweden, Finland, and the United States, including Minnesota, Wisconsin, and Florida. Although these lakes lie in landscapes with little or no human development, they contain fish with Hg levels that pose health risks for human consumption (*1*, *2*), and their sediments show stratigraphic evidence of increasing Hg inputs within the last 100 to 200 years (*3*, *4*). Most researchers have concluded that the Hg contamination must derive from atmospheric pollution.

Despite considerable research on Hg in the environment, the extent to which atmospheric Hg deposition has increased from natural levels is not clear. Reliable measurements of modern Hg deposition are limited to a few years at a few locations (*5–7*), historic deposition rates are essentially unknown, and estimates of the anthropogenic Hg emissions that might be driving increased deposition vary widely (*2*, *8–11*). In remote lakes, it is uncertain how much Hg is deposited directly to the lake surface relative to that delivered to the lake from its catchment, and it is not known whether Hg washed in from surrounding soils is derived solely from atmospheric deposition or from local geologic sources as well.

We addressed these issues by applying a simple mass-balance model to Hg flux data generated from the sediments of seven relatively undisturbed lakes in Minnesota and Wisconsin. Whole-basin Hg accumulation rates are calculated for each lake from multiple (7 to 15) sediment cores that were analyzed stratigraphically for Hg and dated by ^{210}Pb (*12*). By comparing whole-basin Hg fluxes from a group of lakes in a geographic region, we are able to estimate atmospheric deposition rates for modern and preindustrial times and the contribution of Hg from the catchments surrounding the lakes.

Mercury has been measured in sediment cores from other remote or rural lakes (*13*, *14*), but conclusions are limited to a description of Hg accumulation at a single core site and the qualitative statement that atmospheric inputs must have increased. Because sediment deposition patterns and metal concentrations are spatially variable across a lake basin, Hg accumulation rates from a single core cannot be automatically extrapolated to the entire lake (*15*). Whole-basin studies of heavy-metal accumulation (*16*, *17*), including one for Hg (*3*), have been done before but are rare because of the large effort required to analyze and date multiple cores representing the various depositional environments in a single basin. None of these studies used dated cores to calculate explicitly whole-basin metal fluxes for more than one lake.

The study sites include four lakes from the Superior National Forest in northeastern Minnesota (Thrush, Dunnigan, Meander, and Kjostad), Cedar Lake in central Minnesota, Mountain Lake in southwestern Minnesota, and Little Rock Lake in northern Wisconsin (Fig. 1). All of the lakes lie in mixed deciduous-conifer forest, except for Mountain Lake, which is in native tall-grass prairie. Precipitation and wet sulfate deposition increase from west to east across the region (*18*). The lakes are uniformly small and shallow and are fed principally by ground-water seepage and surface precipitation (Table 1). Water residence times are short, except for the two

---

E. B. Swain, Minnesota Pollution Control Agency, 520 Lafayette Road, St. Paul, MN 55155.
D. R. Engstrom, Limnological Research Center, University of Minnesota, Minneapolis, MN 55455.
M. E. Brigham, T. A. Henning, P. L. Brezonik, Department of Civil and Mineral Engineering, University of Minnesota, Minneapolis, MN 55455.

---

*To whom correspondence should be addressed.
†Present address: U.S. Geological Survey, District Office, St. Paul, MN 55101.
‡Present address: James M. Montgomery Consulting Engineers, Wayzata, MN 55391.

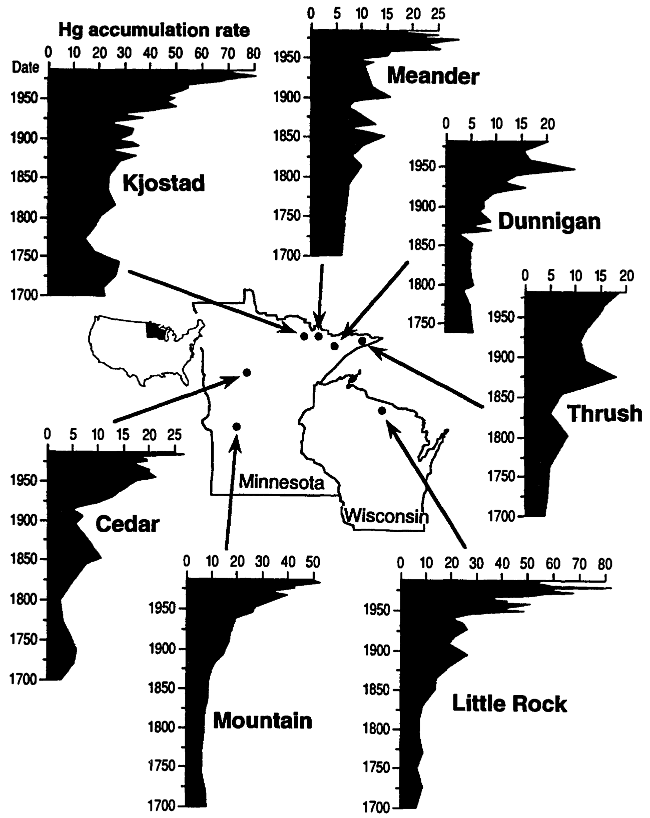

Fig. 1. Stratigraphic increases in Hg accumulation rates (in micrograms per square meter per year) in representative sediment cores from the profundal region of each of the study lakes. The other detailed Hg profiles (not illustrated) are of similar shape and magnitude. Whole-basin increases in Hg accumulation are calculated from these profiles and from an additional 6 to 14 cores from each lake. Chronology and sedimentation rates are provided by ^{210}Pb dating.

western lakes, where much of the inflow is lost through evaporation. The western lakes also have distinctly higher alkalinity than the others, and only Kjostad is noticeably colored by organic acids (30 Pt-Co color units). Apart from logging at the turn of the century, none of the seven watersheds has been appreciably disturbed by land-use changes.

Because this study required a large number of cores, we chose to economize on the number of samples for ^{210}Pb and Hg analysis by sectioning most of the cores at coarse intervals. Historic trends in Hg deposition were provided by a few cores analyzed in stratigraphic detail, whereas the coarsely sectioned cores provided the spatial pattern in Hg accumulation across each basin at a few discrete time intervals (*19*). To estimate whole-basin Hg accumulation, we restricted data analysis to two time-stratigraphic units: preindustrial (before about 1850) and modern (approximately the last decade). We calculated Hg loadings for each lake on an areal basis by weighting the Hg fluxes of each core by the portion of the depositional basin the core represented. Depositional areas were approximated by polygons, and shallow littoral areas lacking fine-grained sediments were excluded.

Detailed profiles of Hg accumulation from each lake show that the flux of Hg to the core sites has increased by a factor of 3 to 4 during the past 140 years (Fig. 1). The data suggest that atmospheric Hg deposition to lakes in this region rose above background at about 1850 and then increased sharply between 1920 and 1950.

On a whole-lake basis, preindustrial sedimentary Hg accumulation rates for the seven lakes ranged between 4.5 and 9.0 μg m^{-2} year^{-1}, and modern rates range between 16 and 32 μg m^{-2} year^{-1} (Fig. 2). Furthermore, Hg accumulation rates increase linearly as the ratio of the land catchment area to the lake surface area increases. The intercept of the observed linear relation can be used to estimate the Hg accumulation rate for a lake with no terrestrial catchment, that is, to predict the net atmospheric deposition rate directly on a lake surface.

The preindustrial atmospheric flux of Hg estimated from the intercept is 3.7 μg m^{-2} year^{-1}, and the modern rate is 12.5 μg m^{-2} year^{-1}. The modern rate is within the range of current measurements of Hg deposition in midcontinental North America. Glass *et al.* (*7*) found a 2-year average of 15 μg m^{-2} year^{-1} in wet deposition for three Minnesota monitoring sites, and Mierle (*20*) measured 10.2 μg m^{-2} year^{-1} in wet deposition for a 1-year study at a catchment in central Ontario. Fitzgerald *et al.* (*5*) reported a mean of about 10 μg m^{-2} year^{-1} for total Hg deposition at one of our sites, Little Rock Lake (6.8 μg m^{-2} year^{-1} for wet deposition and 3.5 μg m^{-2} year^{-1} for dry). Stratigraphic data yield net Hg accumulation, accounting for both wet and dry deposition and losses through evasion of Hg^0 vapor and hydrologic outflows. Evasion was estimated to be 0.7 μg m^{-2} year^{-1} at Little Rock Lake (*5*). Hydrologic outflows are likely to be small for the lakes in our data set—most are seepage lakes with no surface outflows—but data are not available to quantify such losses.

Our estimates of atmospheric deposition in preindustrial and modern times indicate that Hg inputs have increased by a factor of 3.4 in 140 years (3.7 to 12.5 μg m^{-2} year^{-1}); averaging the increase from each lake (Table 1) yields a factor of 3.7, which agrees well with the increase based on the regression intercepts. The 3.7-fold increase translates to an average increase of about 2% per year. Slemr and Langer (*11*) found an annual increase in Hg concentration in air over the North Atlantic Ocean of 1.5% for the period 1977 through 1990. The similarity of these two trends implies that

Table 1. Location and certain morphometric, hydrologic, and chemical characteristics of the lakes investigated, including a comparison of modern and preindustrial Hg accumulation rates for each lake basin.

Lake	Latitude (N)	Longitude (W)	Mean depth (m)	Surface area (ha)	Catchment area* (ha)	Residence time (years)	Alkalinity (μeq liter^{-1})	Hg flux ratio†	Number of cores
Dunnigan	47°42′	91°38′	2.3	32.9	46	5	66	3.58	12
Little Rock	45°50′	89°42′	3.5	18.2	35	10	25	4.03	14
Cedar	47°04′	95°10′	3.8	39.1	88	34	2600	3.37	8
Meander	48°08′	92°09′	4.8	39.6	127	6	70	3.34	11
Thrush	47°54′	90°30′	6.9	6.6	24	6	60	3.30	15
Mountain	45°32′	95°32′	2.7	15.7	82	28	4200	4.88	7
Kjostad	48°07′	92°36′	6.5	167.7	985	5	180	3.22	14

*Catchment area excludes lake surface. †Ratio of modern to preindustrial Hg accumulation rates.

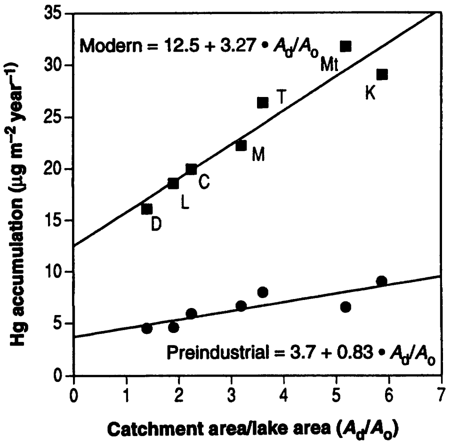

Fig. 2. Whole-basin Hg accumulation rates as linear functions of the ratio of terrestrial catchment area to lake area (A_d/A_o). Modern rates (■) represent the last decade, and preindustrial rates (●) are those before about 1850. The intercept of each regression line predicts the rate of atmospheric Hg deposition to the lake surface, and the ratio of the slope to the intercept estimates the proportion of the atmospheric Hg flux transported to the lake from the catchment (see text). Lake codes are: Dunnigan (D), Little Rock (L), Cedar (C), Meander (M), Thrush (T), Mountain (Mt), and Kjostad (K).

increasing atmospheric concentrations are driving Hg deposition rates and that Hg concentrations in midcontinental air masses are 3.5 to 4.0 times greater than those in preindustrial times (*21*).

Our results show that, for the geographic region represented by the seven lakes, atmospheric Hg deposition has increased by a relatively constant factor and that modern deposition rates are similar among sites (accounting for catchment size). The Hg burden of undisturbed forest soils is also relatively uniform across this region but becomes significantly higher to the east (*22*), presumably because of greater proximity to industrialized regions. Scandinavian researchers have documented a similar gradient of increasing Hg deposition toward industrial areas (*6, 13, 23*).

The slope of the linear relation in Fig. 2 represents the incremental increase in Hg accumulation per unit increase in the ratio between the catchment and lake areas. If all the Hg deposited to the catchment were transported to the lake, the slope would be equal to the rate of atmospheric deposition. On the other hand, if the slope were zero, direct deposition to the lake surface would be the only significant source (*17*). Thus, the slope divided by the atmospheric deposition rate (the intercept) is the proportion of atmospherically deposited Hg transported to the lake from the catchment. This simple model assumes that mineral sources of Hg in the catchment are negligible (*24*), that retention of Hg in the lake is complete, that dry deposition rates are similar for lake surfaces and terrestrial catchments, and that losses of Hg from the lake by evasion or outflow are negligible. The good fit of the data to the linear model suggests that none of these processes exerts a large effect.

Using this model, we find that roughly the same proportion of atmospheric Hg has been transported from the catchments to the various lakes in modern and preindustrial times (26% and 22%, respectively). The balance of the Hg is either volatilized back to the atmosphere or retained by soils in the catchment. Organic matter in soils has a high affinity for Hg (*25*), an affinity that, in contrast to other metals (*26*), may even increase as precipitation pH decreases. In a study in Sweden, Aastrup *et al.* (*27*) found that about 20% of the deposited Hg was transported from the catchment to the lake. By measuring Hg export from three catchments around Harp Lake in Ontario, Mierle (*20*) found that 8 to 16% of deposited Hg was transported to the lake.

Several investigators (*20, 28*) have suggested that because dissolved organic matter (DOM) strongly complexes Hg, the terrestrial export of Hg to lake basins may be controlled by the nature of catchment soils and the movement of organic acids. Our results show that there is no need to account for differing soils among the study lakes. In a given biogeographic region, the humic content of a lake is related to the relative size of the catchment (*29*), an effect for which our model does control. However, because our study encompassed a limited range of DOM values and soil types, we cannot fully evaluate the effect of transport in high-DOM systems.

We conclude that a simple model incorporating the ratio of catchment to lake area can explain most of the variation in Hg accumulation among undisturbed lakes in the upper Midwest of the United States. Atmospheric deposition of Hg can account for all of the Hg accumulating in these remote lakes; local geology appears to contribute minor amounts of Hg. Mercury deposition in this continental area has increased by a factor of about 3.7. This increase suggests that natural atmospheric Hg concentrations were only about 25% of modern levels. Current estimates of recent increases in global atmospheric Hg (*11*) support this conclusion. Moreover, the deposition increase appears to be relatively uniform across our study area, implying regional if not global sources for the Hg falling on these remote sites.

REFERENCES AND NOTES

1. C. D. Wren, W. A. Scheider, D. L. Wales, B. W. Muncaster, I. M. Gray, *Can. J. Fish. Aquat. Sci.* **48**, 132 (1991); M. Verta, *Mercury in Finnish Forest Lakes and Reservoirs* (National Board of Waters and the Environment, Helsinki, 1990); E. B. Swain and D. D. Helwig, *J. Minn. Acad. Sci.* **55**, 103 (1989); R. C. Lathrop, P. W. Rasmussen, D. R. Knauer, *Water Air Soil Pollut.* **56**, 295 (1991); V. W. Lambou *et al.*, *Mercury Technical Committee Interim Report* (Center for Biomedical and Toxicological Research and Waste Management, Florida State University, Tallahassee, 1991).
2. O. Lindqvist *et al.*, *Water Air Soil Pollut.* **55**, 1 (1991).
3. R. D. Evans, *Arch. Environ. Contam. Toxicol.* **15**, 505 (1986).
4. M. Verta, K. Tolonen, H. Simola, *Sci. Total Environ.* **87/88**, 1 (1989); S. A. Norton, P. J. Dillon, R. D. Evans, G. Mierle, J. S. Kahl, in *Acidic Precipitation: Sources, Deposition, and Canopy Interactions*, S. E. Lindberg, A. L. Page, S. A. Norton, Eds. (Springer-Verlag, New York, 1990), vol. 3, pp. 73–102.
5. W. F. Fitzgerald, R. P. Mason, G. M. Vandal, *Water Air Soil Pollut.* **56**, 745 (1991).
6. Å. Iverfeldt, *ibid.*, p. 251.
7. G. E. Glass *et al.*, *ibid.*, p. 235.
8. A. W. Andren and J. O. Nriagu, in *The Biogeochemistry of Mercury in the Environment*, J. O. Nriagu, Ed. (Elsevier, Amsterdam, 1979), pp. 1–21; W. F. Fitzgerald, G. A. Gill, J. P. Kim, *Science* **224**, 597 (1984); J. O. Nriagu and J. M. Pacyna, *Nature* **333**, 134 (1988).
9. J. O. Nriagu, *Nature* **338**, 47 (1989).
10. S. E. Lindberg, in *Lead, Mercury, Cadmium, and Arsenic in the Environment*, T. C. Hutchinson and K. M. Meema, Eds. (Wiley, New York, 1987), pp. 89–106.
11. F. Slemr and E. Langer, *Nature* **355**, 434 (1992).
12. Sediment cores about 1 m long were collected with a piston corer from various depositional regions of the lake bottom. The cores were analyzed at appropriate depth intervals for ^{210}Pb to determine age and sediment-accumulation rates for the past 150 years; ^{210}Pb was measured through its granddaughter product ^{210}Po by alpha-spectrometry methods [J. D. Eakins and R. T. Morrison, *Int. J. Appl. Radiat. Isot.* **29**, 531 (1978)], and dates and sedimentation rates were determined according to the c.r.s. (constant rate of supply) model [P. G. Appleby and F. Oldfield, *Catena* **5**, 1 (1978); M. W. Binford, *J. Paleolimnol.* **3**, 253 (1990)]. Mercury concentrations were determined by cold-vapor atomic absorption spectrophotometry after sediment digestion with a strong acid–permanganate–persulfate technique [Environment-Canada, *Mercury: Methods of Sampling, Preservation, and Analysis: Economic and Technical Review Report EPS 3-EC-81-4* (1981)].
13. K. Johansson, *Verh. Int. Verein. Theor. Angew. Limnol.* **22**, 2359 (1985).
14. M. G. Johnson, L. R. Culp, S. E. George, *Can. J. Fish. Aquat. Sci.* **43**, 754 (1986); S. A. Meger, *Water Air Soil Pollut.* **30**, 411 (1986); R. G. Rada, J. G. Wiener, M. R. Winfrey, D. E. Powell, *Arch. Environ. Contam. Toxicol.* **18**, 175 (1989).
15. J. A. Dearing, *Hydrobiologia* **103**, 59 (1983); J. Hilton and M. M. Gibbs, *Chem. Geol.* **47**, 57 (1984); J. A. Downing and L. C. Rath, *Limnol. Oceanogr.* **33**, 447 (1988); N. J. Anderson, *ibid.* **35**, 497 (1990).
16. R. D. Evans and F. H. Rigler, *Environ. Sci. Technol.* **14**, 216 (1980).
17. P. J. Dillon and R. D. Evans, *Hydrobiologia* **91**, 121 (1982).
18. Mean annual precipitation ranges from 60 cm in southwestern Minnesota to 75 to 80 cm in northern Wisconsin; free surface evaporation decreases from 100 to 50 cm year^{-1} along this same transect. Wet sulfate deposition increases from 5 to 8 kg ha^{-1} year^{-1} in west central and northeastern Minnesota to 15 kg ha^{-1} year^{-1} in northern Wisconsin [*National Atmospheric Deposition Program (NRSP-3)/National Trends Network* (Colorado State University, Fort Collins, 1992)].
19. One to three cores from each basin were analyzed for ^{210}Pb and Hg in stratigraphic detail (intervals of 1 to 2 cm). These detailed profiles guided the collection and sectioning of subse-

quent cores, which were analyzed at three to five contiguous intervals of 5 to 40 cm. The topmost interval gave modern Hg concentrations and fluxes, whereas the bottom interval provided preindustrial values (before 1850 for the Midwest); the middle sections were used to calculate whole-core ^{210}Pb burdens required for dating by the c.r.s. model. The data are in: T. A. Henning, thesis, University of Minnesota (1989); M. E. Brigham, thesis, University of Minnesota (1992); D. R. Engstrom, E. B. Swain, T. A. Henning, M. E. Brigham, P. L. Brezonik, in *Environmental Chemistry of Lakes and Reservoirs*, L. A. Baker, Ed. (American Chemical Society, Washington, DC, in press).

20. G. Mierle, *Environ. Toxicol. Chem.* **9**, 843 (1990).
21. On a global basis, natural and anthropogenic Hg emissions are thought to be roughly equal (*2, 9, 11*). The major natural sources of Hg are volcanoes, continental volatilization, and marine evasion.
22. E. A. Nater and D. F. Grigal, *Nature* **358**, 139 (1992).
23. E. Steinnes and E. M. Andersson, *Water Air Soil Pollut.* **56**, 391 (1991); A. Jensen and A. Jensen, *ibid.*, p. 769.
24. The Hg concentration of the gabbro and granite bedrock in northern Minnesota is low, averaging 10 ng g^{-1} [E. A. Smith, thesis, University of Minnesota (1990)]. The deeper horizons (75 to 100 cm) of the mineral soils in this region contain an average of 14 ng g^{-1} (*22*) compared to an average preindustrial sedimentary Hg concentration of 80 ng g^{-1}. The lake sediments are 30 to 70% mineral matter, which includes diatom silica and authigenic iron as well as detrital silts and clays. Therefore, erosion of mineral soils contributed at most 5 to 12% of the preindustrial sedimentary Hg accumulation.
25. E. Schuster, *Water Air Soil Pollut.* **56**, 667 (1991).
26. M. Lodenius, A. Seppanen, S. Autio, *Bull. Environ. Contam. Toxicol.* **39**, 593 (1987); M. Lodenius and S. Autio, *Arch. Environ. Contam. Toxicol.* **18**, 261 (1989); W. O. Nelson and P. G. C. Campbell, *Environ. Pollut.* **71**, 91 (1991).
27. M. Aastrup, J. Johnson, E. Bringmark, I. Bringmark, Å. Iverfeldt, *Water Air Soil Pollut.* **56**, 155 (1991).
28. K. Surma-Aho, J. Paasivirta, S. Rekolainen, M. Verta, *Chemosphere* **15**, 353 (1986); G. Mierle and R. Ingram, *Water Air Soil Pollut.* **56**, 349 (1991); M. Meili, *ibid.*, p. 719; K. Johansson and Å. Iverfeldt, *Verh. Int. Verein. Theor. Angew. Limnol.* **24**, 2200 (1991).
29. D. R. Engstrom, *Can. J. Fish. Aquat. Sci.* **44**, 1306 (1987); J. B. Rasmussen, L. Godbout, M. Schallenberg, *Limnol. Oceanogr.* **34**, 1336 (1989).

30. We thank M. Hora for initiating the project; W. Popp, H. Wiegner, and D. Verschuren for assistance in the field; D. Helwig for advice in planning parts of the study; and the Legislative Commission on Minnesota Resources, University of Minnesota Water Resources Research Center, and the Acid Deposition Program of the Minnesota Pollution Control Agency for funding. Limnological Research Center contribution 437.

28 April 1992; accepted 25 June 1992

Evidence from 18*S* Ribosomal RNA Sequences That Lampreys and Hagfishes Form a Natural Group

David W. Stock* and Gregory S. Whitt

Lampreys and hagfishes (cyclostomes) traditionally were considered to be a natural (monophyletic) group. Recently, the consensus of opinion, based largely on morphological analyses, has shifted to a view that lampreys are more closely related to jawed vertebrates (gnathostomes) than to hagfishes. Phylogenetic comparisons of 18*S* ribosomal RNA sequences from two hagfishes, two lampreys, a tunicate, a lancelet, and a number of gnathostomes support the monophyly of the cyclostomes. These data force a reassessment of several features of early vertebrate evolution.

Lampreys and hagfishes are the only living vertebrates without hinged jaws. Because the earliest vertebrates in the fossil record also lack jaws, the two living forms have been studied extensively at a number of levels of biological organization in the hope that they have retained features of the earliest vertebrates (*1*). Lampreys and hagfishes appear more primitive than gnathostomes (jawed vertebrates) in a number of features in addition to the absence of jaws, including the absence of paired fins, hard tissues, ribs, a thymus, lymphatic vessels, and genital ducts. These characteristics, along with similarities in the structure of the gills (*2*) and the tongue mechanism (*3*), have led to the traditional view that the two taxa form a natural (monophyletic) group, the Cyclostomata (*4*). However, in a number of respects, hagfishes appear even more primitive than lampreys (*1*). Hagfishes are isosmotic with their marine environment and lack radial muscles associated with the fins, extrinsic eye muscles, nervous regulation of the heart, and any trace of vertebral arches. Because of the absence in hagfishes of these and other characteristics present in lampreys and gnathostomes, a number of researchers have concluded that lampreys are more closely related to gnathostomes than either group is to hagfishes (*1, 5–7*). This phylogeny has also been proposed on the basis of studies of fossil jawless fishes (agnathans) (*8*) and probably represents the consensus of opinion today. A third phylogenetic possibility, that hagfishes are the closest relative of the gnathostomes, has not been proposed seriously. Determining the phylogenetic relationships of lampreys, hagfishes, and gnathostomes is of fundamental importance in testing hypotheses about the order and nature of evolutionary transformation during the earliest stages of vertebrate evolution. For example, different phylogenetic arrangements of these groups have different implications for whether the osmoregulatory strategy of hagfishes is primitive or due to secondary loss. Distinguishing between these alternatives may contribute to the resolution of the question of whether vertebrates originated in marine or freshwater environments (*1*).

A significant problem with many of the morphological and physiological characters that have been used in phylogenetic studies of cyclostome relationships is the difficulty of determining which character states are ancestral (and therefore uninformative with respect to phylogenetic relationships) and which are derived. Character polarity is usually inferred by reference to an assumed outgroup, but for many features (for example, gill structure), it is not possible to make meaningful comparisons with an outgroup because no invertebrate or protochordate group possesses similar structures (*2, 5*). A further problem is the possibility that some proposed primitive features of hagfishes, especially those related to the eye, may actually be the result of more recent degenerative evolution linked to their burrowing habits and life at ocean depths. Molecular sequence comparisons provide a way of overcoming many of the difficulties with outgroup comparison, if the assumption is made that the sequences being used can be aligned unambiguously with protochordate or invertebrate sequences.

The only extensive molecular sequence analyses of cyclostome-gnathostome relationships have used globins (*9*). These analyses have been interpreted as supporting cyclostome monophyly but suffer from a number of limitations. The globin gene family has undergone numerous gene duplications, some of which have been depicted as occurring around the time of the divergences among lampreys, gnathostomes, and hagfishes. Therefore, the phylogeny of the globin genes may not match the phylogeny of the organisms. In addition, globins are short molecules with a relatively rapid rate of evolution (*10*). A slowly evolving molecule is more appropriate for investigating the relationships of these taxa because some of the lineages may have diverged more than 500 million years ago (*11*). The small subunit ribosomal RNA (rRNA) has been used extensively for investigating diver-

---

Department of Ecology, Ethology, and Evolution, University of Illinois, Urbana, IL 61801.

*Present address and to whom correspondence should be addressed: Hopkins Marine Station, Department of Biological Sciences, Stanford University, Pacific Grove, CA 93950.