Reports

Purines and Pyrimidines in Sediments from Lake Erie

Abstract. *Quantitative analyses of purines and pyrimidines in sequential sections of cores from the central and eastern basins of Lake Erie show steeply increasing concentrations in the youngest sediments. This may be related to increased loading of nutrients and recent cultural eutrophication of the lake. The purine and pyrimidine distributions suggest the operation of a specific degradative process for uracil at an extremely early stage in, or prior to, sediment formation.*

In spite of the ubiquity of the purines and pyrimidines in biological material, there have been very few publications on the quantitative distribution of these compounds in sediments. As the repository of genetic information, the purines and pyrimidines are, potentially, of enormous importance in organic geochemistry. Yet we are aware of only one report of purines and pyrimidines in core samples [from the experimental Mohole drilling off Guadalupe Island, Mexico (*1*)] and a number of isolated reports of their occurrence in soils (*2*). Part of the explanation for this scarcity of data may be the lack of an appropriate extraction method, since the usual procedures for extraction of nucleic acid bases from biological material are not applicable to sediments. In addition, reliable and highly sensitive methods for quantitative analyses have not generally been available until fairly recently. To deal with these problems we have made use of an extraction method involving destruction of the mineral matrix, combined with minimum handling of the sample and a highly sensitive and selective method of analysis.

All operations on sediment samples were carried out in Teflon receptacles which could be attached to reflux condensers for solvent extraction or centrifuged. A sediment sample of 5.0 g was weighed out into a receptacle and stirred overnight with 1*M* HCl (35 ml), at room temperature, with a Teflon-coated, magnetic stirring bar. After centrifugation this extract was discarded and the sediment was washed with a second portion of 1*M* HCl (25 ml), which was also discarded. The purpose of this preliminary extraction was to decompose any carbonates which might be present and to eliminate any loosely bound contaminants. The proportion of purines and pyrimidines extracted in this manner was less than 1 percent of that found in the final extraction. Hydrogen fluoride (60 ml, 40 percent) was then added to the residue and the receptacle was heated on a steam bath (under N_2) overnight. The dry residue was extracted with 1*M* HCl (35 ml) under reflux (1 hour), with stirring. After cooling and centrifugation, the supernatant was removed and percolated through a column of activated charcoal (0.6 by 5 cm). The residue was washed with 1*M* HCl (25 ml) and this washing was added to the column. The column was washed with 25-ml portions of 1*M* HCl and H_2O, and purines and pyrimidines were eluted with 40 ml of HCOOH (100 percent). The eluate was evaporated to dryness under vacuum, and the residue was taken up in H_2O (25 ml) and transferred to a column of Dowex 1 X 8 (Cl^-) (0.6 by 2 cm). The column was washed with additional portions of H_2O and the combined eluate and washings (40 ml) were evaporated under vacuum.

Ion-exclusion chromatography was performed on both anion and cation exclusion columns (*3*). The columns used were Aminex A-6 (0.6 by 50 cm) in 0.02*M* NH_4CO_3 plus 0.1*M* NH_3 (*p*H 10.0) and Aminex A-25 (0.6 by 25 cm) in 0.1*M* sodium acetate (*p*H 4.0), both columns operating at 60°C and a flow rate of 24 ml/hour. Detection was carried out by measurement of the optical density of the column efluents at 254 and 280 nm with a Chromatronix model 230 dual channel absorbance detector. Determination of retention times and integration of peaks were done with a Hewlett-Packard 3370B integrator. A more complete description of the methods and some preliminary results have already been reported (*4*). Blank samples carried through the entire procedure demonstrated the absence of significant contamination. The detection limit for an individual purine or pyrimidine was approximately 1 part per billion (10^{-9} g), and the level of contamination was less than 10 parts per billion. An estimate of the recovery efficiency of each base was made by adding standard samples labeled with ^{14}C to sediment samples before the HF digestion.

The core samples studied were collected in late 1972 in Lake Erie by the Canada Centre for Inland Waters. Core LE19 (eastern basin) was collected at 42°33′00″N, 79°53′36″W in a water depth of 63 m. Core LE43 (central basin) was collected at 41°56′06″N, 81°28′42″W in a water depth of 24 m. Both cores were divided into sections of 5 or 10 cm each and freeze-dried. Table 1 shows the concentrations of the purines and pyrimidines that were identified in the surface sections of the cores. The totals found in the surface sediments at these two locations represent 1 to 2 percent of the total nitrogen reported in samples from the same areas (*5*). The very low concentration of uracil is particularly noteworthy. In the sediments of the experimental Mohole no uracil was detected, even in surface samples (*1*). The sedimentation time in oceans is, of course, very much greater than that in lakes, and the possibility exists that organic matter is already devoid of uracil before reaching the bottom. It has been shown, for example, that chlorophylls are almost completely decomposed in Lake Erie and Lake Ontario before

Scoreboard for Reports: In the past few weeks the editors have received an average of 68 Reports per week and have accepted 12 (17 percent). We plan to accept about 12 reports per week for the next several weeks. In the selection of papers to be published we must deal with several factors: the number of good papers submitted, the number of accepted papers that have not yet been published, the balance of subjects, and length of individual papers.

Authors of Reports published in *Science* find that their results receive good attention from an interdisciplinary audience. Most contributors send us excellent papers that meet high scientific standards. We seek to publish papers on a wide range of subjects, but financial limitations restrict the number of Reports published to about 15 per week. Certain fields are overrepresented. In order to achieve better balance of content, the acceptance rate of items dealing with physical science will be greater than average.

Table 1. Concentrations of purines and pyrimidines, in parts per million (ppm), in surface sediments of Lake Erie. Identification of the indicated purines and pyrimidines was based on retention times on standardized anion and cation exclusion columns and on ratios of the absorbance at 254 and 280 nm (*4*). The orders of elution as well as the optical ratios were different on the two columns, and the quantitative results reported are based on analyses in both systems. Average recoveries (percentages) of ^{14}C-labeled purines and pyrimidines added to sediment samples were as follows: cytosine (C), 92; uracil (U), 86; thymine (T), 92; adenine (A), 75; guanine (G), 66; and hypoxanthine (HX), 53. The reported concentrations are not corrected for recovery.

Core	Section (cm)	Concentration (ppm)						
		C	U	T	A	G	HX	Total
LE19	0–10	24	5	16	29	40	9	123
LE43	0– 5	27	5	16	25	29	6	108

reaching the lake bed (*6*). On the other hand, degradation of uracil at the sediment-water interface, where microbial activity is greatest, cannot be ruled out (*7*). Preliminary results on cores sectioned at 1-cm intervals show that uracil is already markedly underabundant in the first centimeter of sediment. Rapid degradation, biological or nonbiological, must be occurring in the water column or at the sediment-water interface.

Figure 1A shows the variation of the total nucleic acid base content with depth in the two cores. The concentration of each base follows, in general, the same profile as the total concentration. The sedimentation rates in the eastern and central basins of Lake Erie, and consequently the ages corresponding to given depths, are quite different. This is indicated by the locations of the *Castanea* and *Ambrosia* horizons, which have been determined in cores taken close to those in the present study (*5*). These horizons represent the depths in the cores in which pollen counts of chestnut (*Castanea*) and ragweed (*Ambrosia*) are observed to decrease and increase, respectively. The decrease of *Castanea* pollen has been correlated with an epidemic fungus disease, which is dated at approximately 1935 for the vicinity of Lake Erie. The increase of *Ambrosia* pollen represents the effect of forest clearance by early colonists and is dated at 1850 (*5*). An approximate relation between depth in the core and age is based on average sedimentation rates for the periods delineated by these horizons, that is, 1850 to 1935 and 1935 to the present. The data in Fig. 1A have been replotted against estimated dates of deposition in this manner in Fig. 1B. The use of average sedimentation rates is, of course, only an approximation made for this analysis, and no corrections have been applied for increasing compaction of the core with depth. In the case of core LE43, dates prior to 1850 (precolonial) have been assigned by assuming the same average sedimentation rate as in the period 1850 to 1935. Consequently, the actual ages of the lower sections of this core are probably considerably greater than indicated.

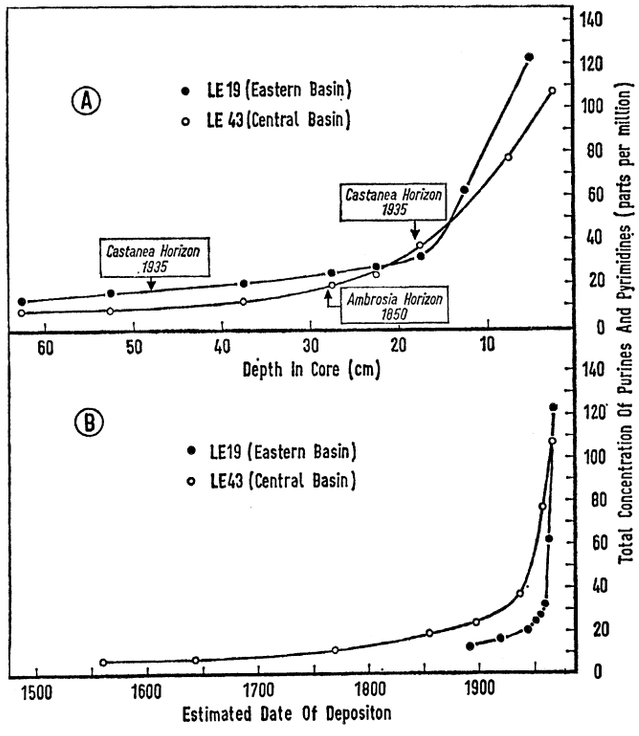

Fig. 1. Total concentration of identified purines and pyrimidines in sediments of the central and eastern basins of Lake Erie (not corrected for losses). (A) Concentration versus depth in the cores; (B) concentration versus estimated date of deposition.

When the total concentrations of purines and pyrimidines are replotted against estimated date of deposition instead of depth in the core, a more revealing picture emerges (Fig. 1B). An extremely rapid increase in purine and pyrimidine contents of sediments of both the central and eastern basins of Lake Erie is seen to have taken place since 1900. The results from the central basin, in particular, are in close agreement with the observations of Kemp *et al.* (*8*). These authors interpreted enrichment of organic C, N, and P as being due mainly to increased loading of nutrients since about 1900. More recent studies suggest that the most important increases in input to the sediments of both basins have occurred since 1950 (*5*). Our data are also in agreement on this point. Although the sedimentation rate is higher at the location of core LE19, the total concentration of extracted purines and pyrimidines is, in general, somewhat lower, except for the upper 15 cm of sediment. It has been suggested that the eastern basin has "aged" less rapidly than other regions of Lake Erie because of its depth, but is (as of 1968) on the verge of becoming eutrophic (*9*). Eutrophication is typified by (among other factors) greatly increased productivity, depletion of dissolved oxygen, an increase in dissolved solids, and an increasing rate of sedimentation of organic material. The data of Fig. 1B, showing a dramatic increase in purine and pyrimidine concentrations beginning around 1950 in the eastern basin, certainly seem to be in agreement with this hypothesis, although rapid alteration of these compounds within the sediment cannot yet be eliminated as an alternative explanation.

W. VAN DER VELDEN
ALAN W. SCHWARTZ
Department of Exobiology,
University of Nijmegen,
Toernooiveld, Nijmegen, Netherlands

References and Notes

1. E. Rosenberg, *Science* **146**, 1680 (1964).
2. See, for example, G. Anderson, in *Soil Biochemistry*, A. D. McLaren and G. H. Peterson, Eds. (Dekker, New York, 1967), p. 67; J. M. Bremner, in *ibid.*, p. 19; W. Flaig, *Soil. Sci.* **111**, 19 (1971).
3. R. P. Singhal, *Arch. Biochem. Biophys.* **152**, 800 (1972); ——— and W. Cohn, *Biochemistry* **12**, 1532 (1973).
4. W. van der Velden, G. J. F. Chittenden, A. W. Schwartz, paper presented at the Sixth International Congress of Organic Geochemistry, Paris, 18 to 21 September 1973.
5. A. L. W. Kemp, T. W. Anderson, R. L. Thomas, A. Mudrochova, *J. Sediment. Petrol.* **44**, 207 (1974).
6. A. L. W. Kemp and C. F. M. Lewis, *Proc. 11th Conf. Great Lakes Res.* (1968), p. 206.
7. A. S. Menon, C. V. Marion, A. N. Miller, *Proc. 14th Conf. Great Lakes Res.* (1971), p. 768.
8. A. L. W. Kemp, C. B. J. Gray, A. Mudrochova, in *Nutrients in Natural Waters*, H. E. Allen and J. R. Kramer, Eds. (Wiley, New York, 1972), p. 251.
9. G. L. Harlow, *Proc. 23rd. Ind. Waste Conf. Purdue Univ.* (1968), p. 856.

10. We thank W. M. J. Strachan and the Canada Centre for Inland Waters, Burlington, Ontario, for the core samples and for supplying valuable background data. We also thank A. L. W. Kemp for advice and critical discussion of the results.

3 April 1974

Beveling of Fine Micropipette Electrodes by a Rapid Precision Method

Abstract. *A technique has been developed for embedding alumina particles 0.05 micrometer in size in the surface of a polyurethane film laid on glass. This abrasive surface is used for rapid, precise, and reliable beveling of Pyrex micropipettes with tip diameters at least as small as 0.1 micrometer. In the snapping turtle retina the beveled electrodes give much better cell penetration and intracellular response stability than unbeveled electrodes of considerably higher electrical resistance.*

The micropipette electrode for intracellular recording has provided a versatile basis for great advances in neurophysiology. Detailed studies, however, have been confined mainly to selected large types of cells, because of the difficulty associated with penetrating small cells without significant damage. The beveling of micropipettes for improved penetration has been reported for tip diameters in the range from 1 to 3 μm (*1*), and Kripke and Ogden (*2*) have recently described a method for beveling tips to final diameters at least as small as 0.3 μm. We report here a rapid technique for the precision beveling of still smaller Pyrex micropipettes. These beveled electrodes have shown great advantages for intracellular recording in the snapping turtle retina, and similar advantages may be expected in many other neural preparations.

Kripke and Ogden (*2*) dispersed 0.05-μm particles of alumina (Al_2O_3) in saline on a glass grinding surface that was rotated with negligible wobble, the electrode being lowered by a micromanipulator while the electrode resistance was monitored continuously. Their technique offers many advantages over earlier methods. We found the slow grinding by the freely floating abrasive, however, to be a major limitation. Kripke and Ogden reported that even the rather short and stiff electrode tip formed by a two-stage puller required about 10 to 15 minutes of grinding. With their technique we found that the longer tip formed by the Livingstone puller, which is used by many workers for producing ultrafine electrode tips, required about an hour. These times are a serious inconvenience because a group of electrodes must be prepared for each experiment, preferably shortly before use to avoid damage to the tip by the contained electrolyte. We thus developed a method for embedding the alumina to form an abrasive surface.

We chose polyurethanes as embedding media because they are unaffected by water or saline, resist mechanical wear, and adhere tightly to glass. The two we have used are Varathane (90-gloss) and Humicure in a 50-50 mixture with its solvent (*3*). Both cure to an extremely smooth surface. Varathane is easy to handle and cures adequately for use within 2 days. Humicure requires thorough mixing with its solvent and must be kept moisture-free until use because its curing is catalyzed by moisture from the air, but it cures overnight to an even harder material than Varathane.

A film of embedding medium was first laid upon the flat surface of a glass beam splitter (Edmund Scientific Company, stock No. 578). These beam splitters are 5 mm thick and hence quite rigid, and their size (67 by 83 mm) is convenient for our purposes. The embedding medium may be laid upon either the coated or the uncoated side, the mirrorlike coated side offering some advantage for visualizing the electrode while one is making contact with the abrasive surface. The surfaces to be coated were thoroughly cleaned, eight to ten drops of embedding medium were placed upon the upturned surface of one beam splitter, and a second beam splitter was placed immediately upon it. The embedding medium was thus squeezed into a thin film between the two surfaces. We then slid the upper beam splitter off with the exertion of a slight upward tension. A thin film of embedding medium was thus laid upon both beam splitters, which were set aside in a dustfree cabinet for initial curing.

The alumina was embedded when the polyurethane was viscous enough to be minimally distorted by contact with the alumina, and to flow minimally into the alumina by capillary action, while still sticky enough to hold the alumina. The optimal initial curing time for Humicure varies with humidity but was about 1 hour in our experience, whereas Varathane required somewhat longer. During embedding the partially cured polyurethane film was faced upward, and a pile of alumina was placed on a clean glass surface held slightly above and to one side of the embedding medium. We used Linde alumina B, with a nominal particle size of 0.05 μm and a specified hardness of 8 on the Mohs scale, Pyrex glass having a lesser hardness of about 5 on that same scale. Gentle air puffs from an empty squirt bottle produced a cloud of powdered alumina, some of which settled upon the polyurethane film, which was then replaced in the dustfree cabinet. When fully cured, it was washed and rubbed by hand to remove all unattached alumina and was ready for use.

Such finely powdered alumina tends to clump, probably because the high surface-to-volume ratio makes electrostatic attractions significant. This was noted by Kripke and Ogden (*2*), and we have also found no way to eliminate this problem entirely. Instead, the clumps of alumina are floated onto the embedding medium only by their own weight. With such light contact and such high viscosity of the polyurethane at the time of embedding, only certain particles of a clump will become embedded, unattached particles being readily removable after the polyurethane has been fully cured. The