

of the lamellae (see Fig. 2). Because of their very small size, the exact composition and crystal structure of the grains has not yet been determined.

Josephinite specimens show evidence of extensive alteration. Serpentine veins crosscut most of the specimens, and serpentine rinds surround nearly all of the specimens. Magnetite occurs as rims around metal grains within specimens and is abundant in the rinds. Iron-nickel metal, apparently secondary, also occurs as veins that crosscut specimens, including those that contain interior Widmanstaetten patterns. The α phases commonly have inclusions of iron cobalt chloride (lawrencite?) that might be an alteration phase. The Widmanstaetten patterns are well preserved only in the central portions of specimens within the rind of serpentine and magnetite, where they were not obliterated by the encroaching metamorphism.

Regardless of the range of temperature or the mechanism of exsolution for the formation of Widmanstaetten pattern described here, the bulk composition requires the homogeneous γ phase from which the phases exsolved to have been above 500°C. These observations are inconsistent with an origin by reduction of sulfides, silicates, or oxides during serpentinization (6). It is more plausible to us that josephinite was derived from the earth's interior and accompanied the peridotite during obduction of the ophiolite (1, 7) and that the alteration features described above were produced at the time of serpentinization of the Josephine Peridotite.

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8. We thank J. I. Goldstein for his helpful discussions. This work was supported in part by research grants from the Office of Naval Research (N00014-78-C-0480) and the National Science Foundation (EAR 78-04387).

5 February 1979; revised 13 July 1979

Evidence of Acidification of Headwater Streams in the New Jersey Pinelands

Abstract. Seventeen years of stream pH data indicate a trend of acidification in two small streams in the New Jersey Pine Barrens which drain relatively undisturbed areas. The decline in pH has amounted to approximately 0.4 unit, with an estimated increase in H^+ concentration of about 50 microequivalents per liter. The data collected to date are consistent with the postulation of an atmospheric source for the increased H^+ .

Acidification of aquatic ecosystems has been observed in the northeastern United States, Scandinavia, and Canada (1-3) and has been linked to acid rain. Recently, concern has been expressed regarding the balance of aquatic communities subject to acidification, particularly with respect to increased concentrations of dissolved aluminum caused by acid precipitation (3). Decreasing stream pH has been reported in Scandinavia, and there appear to have been reversals in that trend from time to time (1); in addition, substantial but ephemeral decreases in stream pH due to snowmelt events have been reported (2). Data which indicate chronic stream-water acidification are rare, however. In most areas of North America where terrestrial and aquatic effects of acid precipitation have been studied, the landscape has been glaciated and is rather young geologically. Reaction with exchangeable cations in the soil and weathering of minerals in the regolith or underlying bedrock tend to neutralize most of the H^+ ;

as a result, the pH of stream water is usually greater than 5 (4) whereas the pH of precipitation is approximately 4 (3).

The Pinelands region, which encompasses approximately 2500 km² of southern New Jersey, is underlain by siliceous sands of Miocene, Pliocene, and Pleistocene age. Soils are mostly Hapludults and Quartzipsamments, which are acid (pH 3.4 to 4.6) and have a very low cation-exchange capacity (0 to 4 meq per 100 g) and percent base saturation (0 to 20 percent) (5). Below the leached A2 horizon, iron oxides coat the mineral grains. There is relatively little neutralization by ion exchange or mineral weathering as precipitation moves through the soil. This low amount of neutralization is evidenced by the low pH of shallow groundwater, which averaged 4.3 for 78 samples collected at McDonalds Branch in 1978 through 1979 (6). This report summarizes data from McDonalds Branch (39°50'N, 74°30'W) and Oyster Creek (39°48'N, 74°15'W), the headwater streams in the Pinelands,

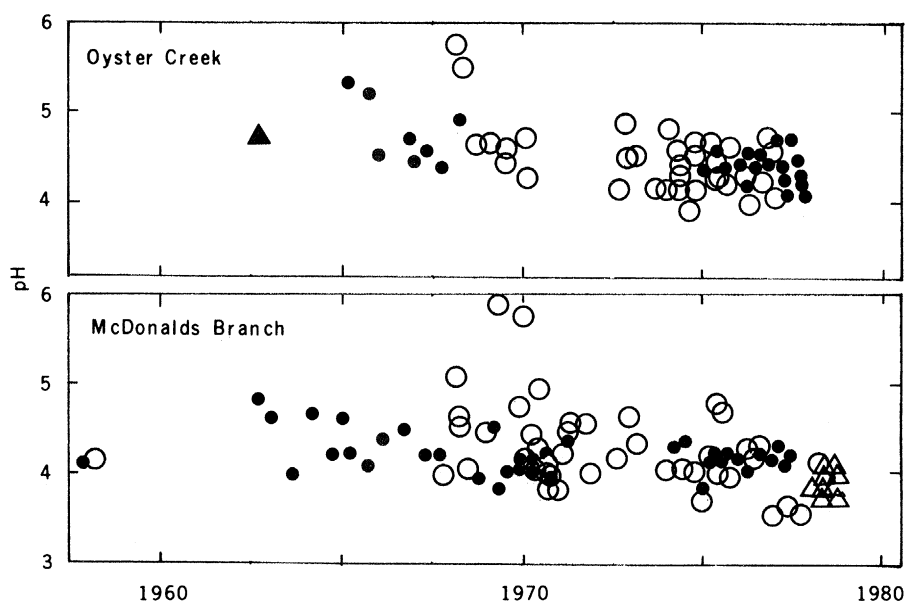


Fig. 1. Stream pH, 1958 through 1979. Closed circles represent samples in which anion and cation equivalents balanced and calculated and measured specific conductances were equal. Open circles are samples for which the chemical analyses were incomplete or for which discrepancies in anion and cation and conductivity balances could not be attributed to errors in pH. The closed triangle represents the average pH determined in a branch of Oyster Creek in a 1963 study (17). Open triangles are monthly means of pH data collected weekly from May 1978 to January 1979 during a University of Pennsylvania trace metal study.

for which there are extensive, long-term pH records.

The McDonalds Branch basin (drainage area, 6.0 km²) and the Oyster Creek basin (drainage area, 19.2 km²) are typical of the Pinelands. They are dominated by pine-oak and oak-pine communities, and the streams are bordered by acid wetlands dominated by Atlantic white cedar (*Chamaecyparis thyoides*) and sphagnum moss (*Sphagnum* sp.). There is little human activity in the McDonalds Branch watershed. There are a few dirt roads, and the litter of the forest floor is burned periodically to reduce the hazard of destructive forest fires. There are some houses, summer camps, and abandoned cranberry bogs in the Oyster Creek watershed.

Stream water has been collected on a random schedule since 1963, principally by the U.S. Geological Survey; usually 2 to 12 samples were collected each year. There was no seasonal bias in sampling McDonalds Branch. There was a slight bias in Oyster Creek samples in that the spring months were represented by more samples than other periods of the year. This effect was consistent throughout the period of record. Stream pH was monitored weekly from 1978 through 1979 for a trace metal study conducted by the University of Pennsylvania (7). All pH values were measured with a glass electrode.

The stream water is acid and yellow in color, particularly in summer, as a result of organic matter (8). The streams have a mixed-cation, mixed-anion character and a very low content of dissolved solids, usually in the range 15 to 35 mg/liter (9). Figure 1 shows the pH measured during the period of record. I analyzed the data to determine the accuracy of the pH measurements by comparing calculated and measured specific conductance and the sums of anion and cation equivalents. In most of the samples H⁺ dominated the specific conductance because of its relatively high concentration and very high equivalent conductance. Thus a relatively small error in measured pH (0.1 to 0.2 unit) causes a substantial discrepancy between measured and calculated specific conductance. The pH data (Fig. 1) are presented in groups in which I have varying levels of confidence (10). Regressions of pH on time are summarized in Table 1. The results indicate a decrease in pH since 1963 which has amounted to an increase in H⁺ concentration of 26 to 80 meq/liter.

Several possible causes which might lead to acidification were investigated. One possibility is the acidification of pre-

Table 1. Regressions of stream pH on time; *N* is the number of samples, *r* is the correlation coefficient, and *P* is the level of significance; *a*₀ and *a*₁ are coefficients in the regression $pH = a_0 + a_1x$, where *x* is the number of months after June 1963.

Data source	<i>N</i>	<i>a</i> ₀	<i>a</i> ₁	<i>r</i>	<i>P</i>	Δ μeq H ⁺ per liter (1963– 1978)
<i>McDonalds Branch</i>						
USGS data, 1963–1978	90	4.42	−0.0022	−.22	.05	+57
USGS data + UP data*	100	4.49	−0.0030	−.32	.01	+80
USGS data, anion equivalents balance cation equivalents; measured and calculated specific conductances are equal	36	4.35	−0.0012	−.29	n.s.†	+29
<i>Oyster Creek</i>						
All USGS data	78	5.10	−0.0047	−.56	.01	+48
USGS data, anion equivalents balance cation equivalents; measured and calculated specific conductances are equal	26	4.89	−0.0027	−.53	.01	+26

*Includes all data collected by the U.S. Geological Survey (USGS) from 1958 to 1978 and the monthly average pH of University of Pennsylvania (UP) samples. †Not significant.

cipitation during the last two decades, as described for other areas in the northeastern United States (11, 12), and there are some precipitation pH data which suggest such a trend in southern New Jersey. Precipitation samples collected at several sites in the Mullica and Cedar Creek basins in 1970 through 1972 had an average pH of 4.4 (13), and samples collected near Oyster Creek for 7 months in 1972 had an average pH of 4.25 (12). From May 1978 to April 1979 the average pH of weekly precipitation samples at McDonalds Branch was 3.9. The SO₄^{2−} concentration was correlated with the H⁺ concentration over the period in both McDonalds Branch and Oyster Creek (14), which is consistent with the hypothesis of an atmospheric source of acid since SO₄^{2−} is the main anion balancing H⁺ in precipitation in the Northeast (11). There are not, however, significant increases in strong acid anions (SO₄^{2−}, NO₃[−]) which balance the increase in H⁺ in the stream water.

Oxidation of sulfides in the cedar bogs to form H₂SO₄ is possible during periods in summer when the water table is low, but evidence is lacking to support the contention that oxidation of geological sulfides caused the long-term trend of acidification (15). There is no evidence that changes in land use or periods of prescribed burning affected the stream pH. Likewise, there is no consistent evidence to date which indicates that changes in the yearly precipitation amounts were related to pH changes or that biological acidification in the bogs increased over the period.

It appears that the decrease in stream pH is a real phenomenon and not attributable to differences or bias in sampling

or measurement. McDonalds Branch and Oyster Creek are extremely acid, and pH values of less than 4, which were rare before 1970, are quite common at present. A number of other small streams in the region have been sampled since 1975, and these also frequently have a pH of 4 or less (16).

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8. Essentially all of the colored material passes through a 100-Å membrane filter.
9. The dominant dissolved constituents were Ca²⁺, Mg²⁺, Na⁺, K⁺, SO₄^{2−}, Cl[−], and SiO₂. These concentrations and other chemical parameters were measured in most of the samples by the U.S. Geological Survey. These data were obtained from the U.S. Geological Survey Water Resources Division, Trenton, N.J.
10. Samples in which the anion equivalents balance the cation equivalents (to within 15 percent) and the calculated conductance equals the measured specific conductance (to within 15 percent) are considered most reliable. Eleven samples from McDonalds Branch were excluded from Fig. 1 on the basis that the contribution of the measured H⁺ to the calculated specific conductance exceeded the measured specific conductance.
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14. For McDonalds Branch, $\text{SO}_4^{2-} = 1.36 \text{ H}^+ + 62.6$, $r = .66$ (significant at $P = .01$), $N = 36$; for Oyster Creek, $\text{SO}_4^{2-} = 0.9 \text{ H}^+ + 78.1$, $r = .44$ (significant at $P = .05$), $N = 26$. Concentrations are in microequivalents per liter; r is the correlation coefficient; N is the number of samples in which anion and cation equivalents and calculated and measured specific conductances agree.

15. The summer pH was higher than the winter pH, and there was no relationship between discharge and pH. There was no tendency for pH to decrease during high flow events in summer.
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25 January 1979; revised 10 September 1979

Lens Epithelial Cell Elongation in the Absence of Microtubules: Evidence for a New Effect of Colchicine

Abstract. Embryonic chick lens epithelial cells cultured in serum-supplemented medium elongated in the absence of microtubules after treatment with the anti-microtubule drug nocodazole. Colchicine, at concentrations lower than those that dissociate microtubules, blocks cell elongation and the associated increase in cell volume. These results indicate that an increase in cell volume, not microtubules, is responsible for lens cell elongation and suggest a previously undescribed effect of colchicine on cell volume regulation.

Microtubules (MT's) are nearly ubiquitous cell organelles that have been associated with a wide range of cellular activities including intracellular transport (1), regulation of membrane function (2), maintenance of cell shape (3), ciliary and flagellar motion (4), chromosome movement (5), and active cell shape change (6-8). Much of the evidence for the role of MT's in these processes has come from the use of colchicine or drugs that, like colchicine, lead to the depolymerization of cytoplasmic MT's. Although it is likely that MT's play a central role in many of these activities, investigators have cautioned that in some cases the treatments used to interfere with MT structure may actually be exerting their disruptive activity through effects on other cellular structures (8, 9). We have found evidence which suggests that the ability of colchicine and three other anti-MT drugs to block lens epithelial cell elongation in vitro during lens fiber cell differentiation is due to their effect on cell volume regulation rather than their

direct effect on cytoplasmic MT's.

Embryonic chick lens epithelial cells cultured in medium supplemented with fetal calf serum (FCS) elongate and differentiate into lens fiber cells (8, 10). The early phase of this elongation, in which the cells approximately double in length, can occur during nearly complete inhibition of protein synthesis (11). Lens epithelial cell elongation is blocked by colchicine (11) and the anti-MT agents vinblastine (10), demecolcine (Colcemid) (12), and podophyllotoxin (12). Colchicine ($2 \times 10^{-5}M$) does not, however, inhibit specialization of lens epithelial cells for δ -crystallin protein synthesis or the accumulation of δ -crystallin messenger RNA, events that are associated with lens fiber cell differentiation (10, 13, 14).

We have measured the volume of elongating embryonic lens epithelial cells and cells prevented from elongating with $10^{-6}M$ colchicine. Central lens epithelia from 6-day-old chick embryos (15) were cultured in 35-mm Falcon plastic tissue culture dishes as previously described

(10, 16). Cell volume was determined after 0, 5, and 24 hours of culture by multiplying the mean cell length (17) of central epithelial cells by their mean area (18). Control and colchicine-treated lens epithelial cells had a similar mean cell area at 0, 5, and 24 hours of culture (18). Epithelial cells cultured in FCS without colchicine elongated from $10.0 \pm 0.2 \mu\text{m}$ [mean \pm standard error (S.E.)] at 0 hours to $19.1 \pm 0.4 \mu\text{m}$ at 5 hours and $26.6 \pm 0.6 \mu\text{m}$ after 24 hours. This resulted in a mean volume increase during elongation that was proportional to cell length, as shown in Fig. 1A. Colchicine ($10^{-6}M$) completely blocked the increase in cell length and the associated increase in cell volume (Fig. 1A).

We then tested the ability of other MT-depolymerizing drugs to inhibit lens epithelial cell elongation, hoping to find one that would permit discrimination between the effects of these agents on cytoplasmic MT's and their possible direct effect on cell volume. One of these drugs, nocodazole, a synthetic MT-depolymerizing agent that binds to the colchicine binding site of tubulin (19) and is active in vivo (20) and in vitro (19), did not prevent FCS-stimulated lens cell elongation at either 1 or 10 $\mu\text{g/ml}$, although it blocked mitosis at both concentrations (21). Figure 1B shows the extent of lens epithelial cell elongation in the presence or absence of nocodazole at 10 $\mu\text{g/ml}$. Electron microscopic examination of lens epithelial cells cultured for 5 to 24 hours showed that, in contrast to controls (Fig. 2A), nocodazole-treated elongated cells completely lacked cytoplasmic microtubules (Fig. 2B). Nocodazole-treated cells also contained prominent bundles of 10- to 12-nm filaments similar to those described in other cell types treated with anti-MT agents (20, 22). Except for these differences, drug-treated cells were similar in morphology to untreated controls (23).

Additional evidence suggesting that colchicine does not prevent lens cells elongation by disrupting the structure or function of MT's was obtained by examining the effect on lens cells of low concentrations of colchicine. Lens epithelial cell elongation was blocked at $10^{-8}M$ colchicine and partially inhibited at concentrations as low as $10^{-10}M$. Colchicine associates with chick brain tubulin with a binding constant of 1×10^6 to 2×10^6 liter/mole (24). Assuming that the binding constant for chick lens tubulin is similar to that for chick brain tubulin, only a small fraction of the tubulin molecules would be bound to colchicine at drug concentrations that block lens cell elon-

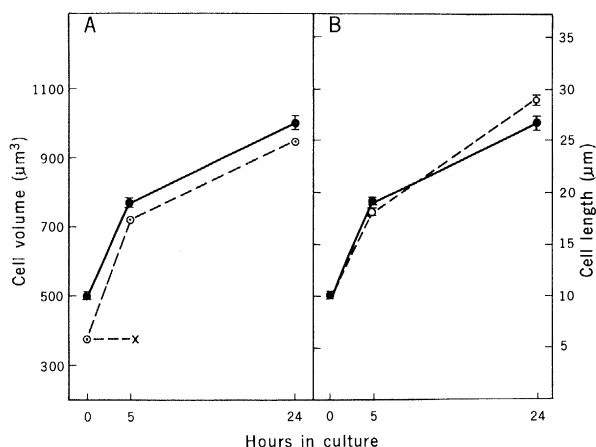


Fig. 1. (A) (●) Mean length (\pm S.E.) and (○) volume of 6-day-old embryonic chick lens epithelial cells cultured for 0, 5, or 24 hours in medium supplemented with FCS. (x) Average volume of epithelia cultured for 5 hours with FCS and $10^{-6}M$ colchicine. (B) Mean cell length of epithelia cultured as in (A) in the (○) presence or (●) absence of nocodazole (10 $\mu\text{g/ml}$).