Phosphate in Interstitial Waters of Anoxic Sediments: Oxidation Effects during Sampling Procedure

Abstract. *Oxidation during sampling procedures significantly decreases the inorganic phosphate concentrations of interstitial water rich in iron (II). All sampling and analytical procedures must be carried out in an inert atmosphere. Orthophosphate in the interstitial water of Cheaspeake Bay sediments, in equilibrium with vivianite, is a potential nutrient source for the overlying water.*

Phosphate concentrations in interstitial water and exchange between the sediment–interstitial water system and the overlying water are important considerations for the phosphorus budgets of aqueous environments. Accurate analytical values are necessary for an understanding of this system. We have investigated the distribution of phosphate in the interstitial water of Chesapeake Bay sediments by using the analytical method outlined by Harwood *et al.* (*1*).

Sampling procedures have been reported to cause changes in the concentration of dissolved constituents in interstitial water (*2–4*). We examined our sampling and analytical procedure for phosphate to determine whether there were comparable systematic errors. In studies of the trace element composition of interstitial water in anoxic sediments, no serious effects of oxidation reactions on the concentration of dissolved species have been reported (*5*). We encountered considerable interference in the investigation of iron (II) in interstitial water because of oxidation during the sampling procedure (*4*). Small amounts of oxygen trapped within the squeezer barrels and the filters oxidize iron (II) in the first few milliliters of expressed interstitial water. This lowers the analytical iron (II) concentration. As oxygen within the squeezer is used up, the concentration in successive portions of the interstitial water rises and finally attains a constant value. Under comparable conditions of oxidation we observed similar behavior for phosphate concentrations. We therefore investigated the role of oxidation reactions in altering the phosphate concentration of interstitial water during handling and squeezing operations.

In field tests, ten cores were taken at a station in the upper Chesapeake Bay (38°56′N, 76°25′W) with a Benthos gravity corer. A layer 5 cm thick, 70 cm below the top of each core, was extruded into a plastic bag, manually homogenized, and then loaded into two 2.5-cm gas-operated nylon squeezers (*6*) containing filters deaerated in the manner described by Troup *et al.* (*4*). The entire process was carried out in glove bags filled with nitrogen. Subsequent sampling and analytical operations were carried out in nitrogen for the first of each of the duplicate pairs of sediment samples and in air for the second, after the second squeezer had been removed from the bag, opened, and exposed to the atmosphere for 15 minutes to simulate previous rapid handling techniques. Squeezing began simultaneously for each pair, and the first 13 to 15 ml of expressed water was collected. One milliliter from each of these samples was pipetted into the mixed reagent for colorimetric analysis (*1*). For each of the ten pairs the concentration of soluble reactive phosphate for the sample exposed to air was lower than that for the unexposed sample. The mean of the differences was statistically different from zero at the 99.5 percent confidence limits.

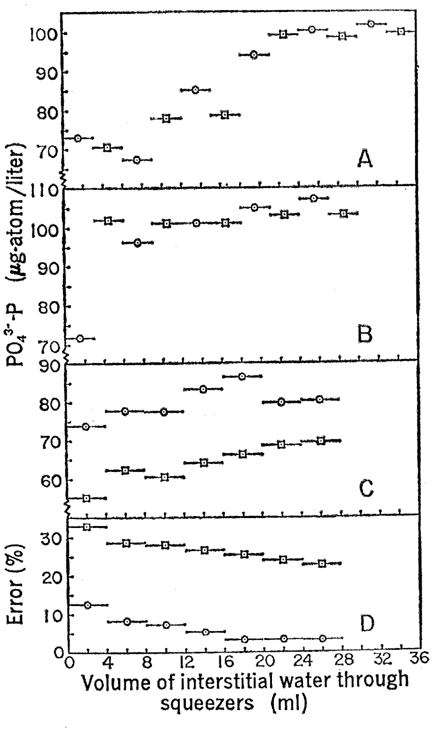

Fig. 1. (A) Change in phosphate concentration as interstitial water is squeezed through standard filters in a nitrogen environment; (circles) first duplicate; (squares) second duplicate. (B) Change in phosphate concentration as interstitial water is squeezed through deaerated filters in a nitrogen environment; (circles) first duplicate; (squares) second duplicate. (C) Change in phosphate concentration as interstitial water is squeezed, with (circles) total protection against oxidation and (squares) no protection against oxidation. Each data point is the mean of duplicate determinations. (D) Cumulative percentage error for squeezing, with (circles) total protection against oxidation and (squares) no protection against oxidation. See text for explanation.

We investigated this oxidation phenomenon further in experiments on cores returned to the laboratory and stored at 4°C. In each experiment duplicate sediment samples were used and all manipulations were performed in a nitrogen-filled bag. In the first experiment we tested standard filters and in the second, performed on a different core, deaerated filters. Consecutive 3-ml portions were collected, and analyses for phosphate and iron (II) (*7*) were made on alternate portions. A comparison of the phosphate results in Fig. 1, A and B, demonstrates the effect of deaerating the filters on the shape of the "squeezing curve." In the third experiment we evaluated the overall effect of the combined sources of oxidation by using a single sample of homogenized sediment. Four squeezers were filled with samples of this sediment. One set of duplicates was squeezed under nitrogen with deaerated filters, while the second set of duplicates was squeezed in air with standard filters after a 15-minute exposure to the atmosphere. Phosphate and iron (II) determinations were made on alternate 3-ml and 1-ml portions. Each phosphate value in Fig. 1C represents the mean of two overlapping duplicates. These results indicate the importance of protecting against oxidation for the analysis of phosphate in interstitial water rich in iron (II). Usual sampling procedures involve collecting all of the interstitial water that can be expressed in a reasonable amount of time and performing analyses on the pooled water sample. The effect of oxidation on the phosphate concentration in such a cumulative sampling procedure is demonstrated in Fig. 1D, which shows the cumulative percentage error for 4-ml increments if the "true" concentration is represented by the mean of the last four values for the unexposed sample.

In all but one of the laboratory tests the maximum iron (II) concentrations ranged from 100 to 300 μg-atom/liter. On the one occasion when the iron (II) concentration did not exceed 25 μg-atom/liter only a very slight squeezing curve was observed with total oxidizing conditions, and no curve was observed with total nonoxidizing conditions. We conclude that for interstitial water rich in iron (II) small quantities of oxygen present in the filters and trapped in the squeezers during handling oxidize the iron (II) as it is squeezed until all of the oxygen is depleted. The iron (III) thus produced removes inorganic phosphate from solution either by precipitating it directly as an iron phosphate, or by first precipitating as iron hydroxide and subsequently scavenging phosphate from solution by sorption. The slight squeezing curve observed when nonoxidizing conditions were used is probably due to incomplete elimination of oxygen from the system. Trace quantities of oxygen in the bulky glove bag and the water-pumped nitrogen are the major sources of contamination.

Using protection against oxidation, we investigated several other factors that could cause systematic errors in the phosphate determination. Within the overall limits of our analytical procedure, ± 10 percent, there were no significant differences in the phosphate concentrations in interstitial water in tests comparing Millipore, Gelman, and Nucleopore filters and pore sizes of 0.22, 0.10, 0.05, and 0.025 μm. Similarly, a comparison of the results obtained with squeezing at 7° and 20°C and at 50 and 200 pounds per square inch (~ 250 and 1000 cm-Hg) showed no observable differences in the phosphate concentrations. There was a slight phosphate enrichment for both fresh and saline standard phosphate solutions percolated through the filters. This apparent elution of phosphate from the filters is consistent with the observations of others (*3*, *8*). However, the only factor significantly affecting the phosphate concentration is the oxidation of iron (II) in samples rich in iron (II).

Using the procedures described above, we examined the interstitial water from ten cores taken in Chesapeake Bay during August 1972 from deepwater stations in or near the main channel along the axis of the bay. Sections of these cores down to 100 cm were immediately analyzed on board the R.V. *Ridgely Warfield.* Phosphate determinations were made on portions of the water obtained after 10 to 15 ml had been squeezed. The depth profiles of the soluble reactive phosphate concentration for the deepwater stations (Fig. 2A) show a general increase downstream for the upper 1 m of sediments to about mid-bay (station 818), after which there is a considerable decrease. However, for most samples a well-developed concentration maximum between 10 and 30 cm is observed.

The predominant phosphorus species in these interstitial waters is orthophosphate, a form readily usable by phytoplankton if brought to the surface waters. Organic phosphate compounds that may be labile under the analytical conditions may account for a few percent of the observed concentrations (*1*). Except for this we have found no significant quantity of dissolved organic phosphate capable of being oxidized by intense ultraviolet radiation. Tests for the presence of arsenic (*9*) showed less than 1 percent interference with the phosphate analysis.

The possible significance of the observed phosphate concentrations, which average 1000 times the relatively uniform value of 0.2 μg-atom/liter in the bay water (*10*), can be demonstrated by a few calculations. The total phosphate content in the interstitial water of the upper 20 cm of sediments, for which we have determined an average porosity of 86 percent and an average phosphate concentration of 100 μg-atom/liter, is 1.72 μg-atom/cm². The total phosphate content of the overlying water column, with an average depth of 8.5 m (*11*) and an average

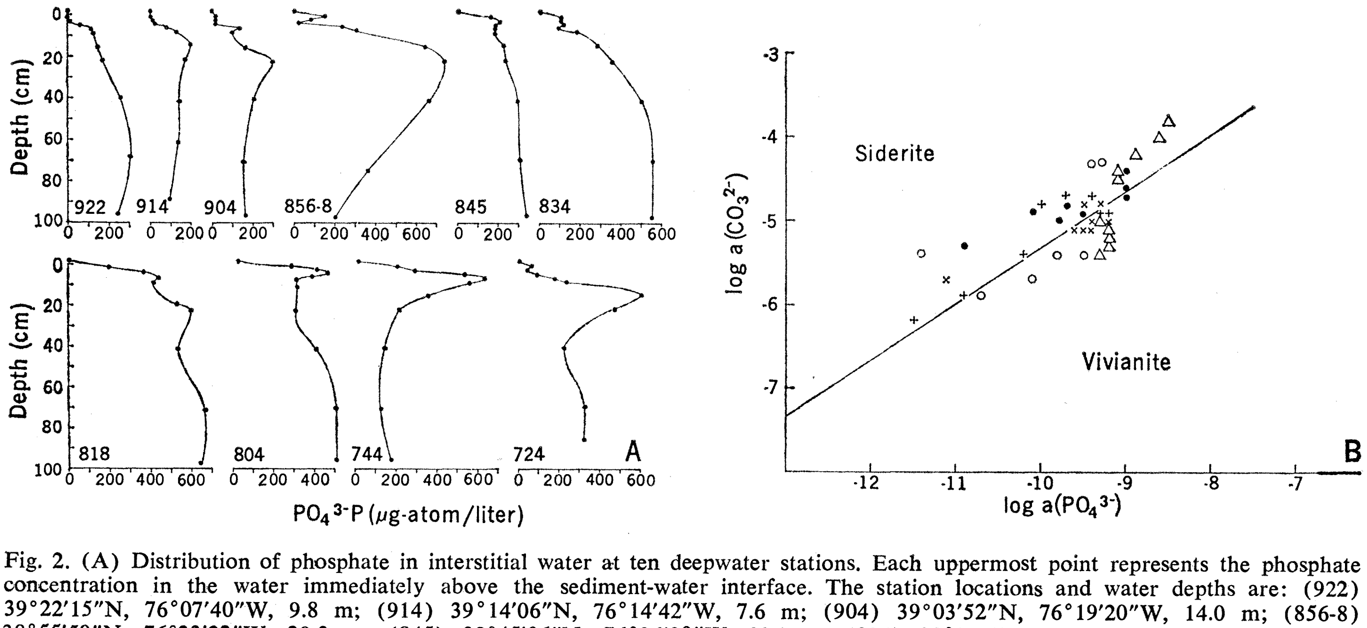

Fig. 2. (A) Distribution of phosphate in interstitial water at ten deepwater stations. Each uppermost point represents the phosphate concentration in the water immediately above the sediment-water interface. The station locations and water depths are: (922) 39°22′15″N, 76°07′40″W, 9.8 m; (914) 39°14′06″N, 76°14′42″W, 7.6 m; (904) 39°03′52″N, 76°19′20″W, 14.0 m; (856-8) 38°55′59″N, 76°23′22″W, 29.3 m; (845) 38°45′06″N, 76°26′00″W, 29.3 m; (834) 38°33′50″N, 77°26′33″W, 24.4 m; (818) 38°18′48″N, 76°13′35″W, 33.5 m; (804) 38°04′48″N, 76°12′38″W, 30.5 m; (744) 37°45′12″N, 76°11′28″W, 29.9 m; (724) 37°23′36″N, 76°04′42″W, 21.3 m. (B) Equilibrium between siderite and vivianite. The straight line is the theoretical siderite ($FeCO_3$)-vivianite [$Fe_3(PO_4)_2 \cdot 8H_2O$] phase boundary. The logarithms of the activities (*log a*) were calculated from the concentrations by the ion pairing model (see text). (Crosses) Station 922; (pluses) station 914; (filled circles) station 856-8; (triangles) station 804; (open circles) station 724.

concentration of 0.2 μg-atom/liter, is 0.17 μg-atom/cm^2. Consequently, a catastrophic overturn of the top 20 cm of sediment by storm surge, slumping, or dredging operations would liberate into the water column more than ten times its normal content of inorganic phosphate. In the upper quarter of the bay there appears to be sufficient soluble iron in the sediments to precipitate all of this phosphate. In the lower three quarters of the bay, however, this is not the case; there is sufficient soluble iron to remove only about 25 percent of the phosphate in this manner.

The rather large gradients in phosphate concentration in the upper 10 to 20 cm indicate a possible upward diffusive flux of phosphate into the overlying water. If we assume that the concentration gradient is linear with an average value of 15 μg-atom/liter per centimeter and estimate the diffusion coefficient to be 10^{-6} cm^2/sec (*12*), we calculate an upward diffusive flux of 9×10^{-3} μg-atom/cm^2 per week. This would amount to an addition of slightly more than 5 percent of the total phosphate content of the water in a week. These few calculations indicate the amount and potential availability of phosphate in the most accessible portion of the sedimentary nutrient reservoir.

We have been interested in the mechanisms within the sediment–interstitial water system that control the interstitial phosphate concentrations. Approaching this problem from a thermodynamic point of view, we calculated from published inorganic stability constants (*13*) the speciation of the free and complexed forms of phosphate, iron (II), and carbonate. Appropriate activities for these species were determined from the product of the calculated free ion concentrations and Debye-Hückel activity coefficients (*14*). The logarithmic activities for carbonate, phosphate, and iron (II) calculated from our data on interstitial water for the entire length of the bay correlate well with those for the siderite-vivianite equilibrium phase boundary (Fig. 2B). These results and the presence of siderite and vivianite as mineral phases in these sediments, as suggested by our preliminary x-ray data, indicate an apparent equilibrium among the three soluble species and two solid phases. The results of Troup *et al.* (*4*), which describe the codependent distribution of carbonate, phosphate, and iron (II) in the interstitial water of the upper bay, are thus extended to include the interstitial water of the lower bay, in which the maximum iron (II) concentrations are an order of magnitude lower.

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Postlesion Axonal Growth Produces Permanent Functional Connections

Abstract. *Lesions of the entorhinal cortex in neonatal rats cause the commissural projections to the dentate gyrus to spread from their normal location in the inner molecular layer into the outer molecular layer, a region normally occupied by afferents from the entorhinal cortex. These lesions also cause the short-latency response to commissural stimulation to spread into the outer molecular layer, a result suggesting that these abnormally located connections are operative.*

Neurons in the mammalian brain which normally interconnect to form intricate circuits are now known to form new connections following brain lesions. These effects are particularly dramatic when lesions are made in developing animals. The types of changes observed include (i) rerouting of a fiber path to a different cell population (*1*), (ii) spread of an afferent system into areas of a given dendritic field that it would not normally occupy (*2*), and (iii) sprouting of synaptic connections within an area normally innervated (*3*). Whether or not any of these forms of postlesion growth result in the formation of permanent functional synaptic contacts is unknown. Studies in the peripheral nervous system have suggested that abnormal innervation of muscle can produce synapses that appear structurally perfect but are rendered inactive when normal innervation is reestablished (*4*). Thus, it is possible that the abnormally located synaptic contacts that form in brains as a result of lesions are never operative or are suppressed or retracted, possibly as part of the recovery of function phenomenon. This study provides strong evidence that postlesion axonal growth results in the formation of permanent functional contacts.

We have reported anatomical evidence that removal of the entorhinal cortex in rats 11 days old causes the commissural projections to the granule cells in the dentate gyrus to greatly increase and occupy much of the space made available by the lesion (*2*). In the normal adult rat, entorhinal projections to the dentate gyrus occupy the outer molecular layer (Fig. 1a) (the molecular layer contains the granule cell dendrites of the dentate gyrus), while the commissural terminals innervate exclusively the inner molecular layer (Fig. 1b). The two projections thus are located in adjacent layers on