Reports

Phosphorus Dynamics in Lake Water

Abstract. *An exchange mechanism exists in lake water between phosphate and plankton, but the excretion of an organic phosphorus compound by the plankton is also a significant process. It results in the extracellular formation of a colloidal substance, and most of the nonparticulate phosphorus in lake water is in this form.*

Phosphorus has been shown to contribute to the eutrophication of many lakes. However, the species of soluble phosphorus compounds and the mechanisms by which they are produced have not yet been identified (*1*). Until this is done, it will not be possible to understand how phosphorus compounds might influence lake productivity.

I used kinetic studies of radioactive phosphate in conjunction with gel filtration to identify the biologically important forms of phosphorus in lake water and the rate constants for their formation. These experiments revealed that a steady state is rapidly attained between radioactive phosphorus and lake water in the summer with the following composition: a particulate fraction [the fraction removed when lake water is filtered through a 0.45-μm filter (Millipore), which includes algae, bacteria, and nonliving particulate matter] containing the bulk of the phosphorus plus small amounts of a soluble high-molecular-weight organic phosphorus compound, a low-molecular-weight organic phosphorus compound, and soluble inorganic phosphate, the turnover time of which is very short. An exchange mechanism predominates between the inorganic phosphate and the particulate fraction, but some phosphorus is excreted by the microorganisms in the form of the low-molecular-weight compound. Polycondensation of the low-molecular-weight compound produces the high-molecular-weight compound and also releases phosphate which then becomes available for uptake by the plankton.

Experiments were conducted on samples of water from the upper 2 m of a small eutrophic lake (Heart Lake) 96 km northwest of Toronto, Ontario (*2*). The data presented here typify those of the summer to early fall period. Radioactive phosphate ($[^{32}P]PO_4$) was added to lake water, and then 5-ml subsamples were passed through Millipore filters (filter diameter, 25 mm; pore size, 0.45 μm). The logarithm of the percentage of radioactivity in the filtrate is plotted as a function of time in Fig. 1. Within 1 minute half of the isotope was removed by membrane filtration, and in less than 1 hour an asymptote (the constant percentage distribution of radioactive phosphate between the particulate and filtrate forms) was reached that persisted for at least another 24 hours. At the asymptote level (about 2 percent) the rate of uptake of the isotope must equal the rate of loss. If these data were consistent with a simple two-compartment exchange model, that is, a particulate form exchanging with phosphate, a straight line should result when the asymptote level is subtracted from the data points (*3*). Instead of a straight line, two lines of different slope were obtained. These data suggest that more than two compartments are involved.

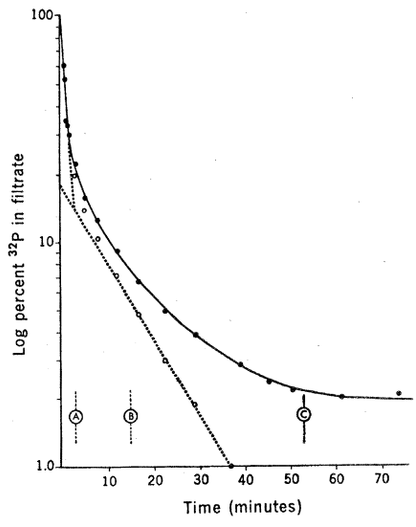

Fig. 1. Changes in the percentage of ^{32}P in the filtrate. Concurrent Sephadex gel filtration analyses were made at points *A*, *B*, and *C*: percentage of ^{32}P in the filtrate at time t (●); percentage of ^{32}P in the filtrate at time t minus percentage of ^{32}P in the filtrate when the steady state is reached (○).

I identified the forms of ^{32}P in the filtrate by injecting 2.5 ml of the filtrate into a column (34 by 2.5 cm) packed with Sephadex G-25 and eluting it with 0.3 percent NaCl and 0.02 percent sodium azide (NaN_3). It was not necessary to concentrate the filtrate, and no retention of radioisotope by the columns was found (*4*).

Three minutes after the isotope had been added to whole lake water 79 percent of the phosphorus had been removed by filtration, but already there were three forms of ^{32}P in the filtrate: one peak corresponded to that for phosphate ($[^{32}P]PO_4$), one had an apparent molecular weight of 250 (an unidentified material which will simply be referred to here as XP), and the third exceeded the fractionation range for Sephadex G-25. When Sephadex G-75 and Sepharose 4B columns were used, this third form exceeded the fractionation range of 5,000,000 (for polysaccharides). Using sucrose density gradient ultracentrifugation (*5*), I confirmed that this fraction was a macromolecule or aggregate, not merely something excluded by Sephadex. (It will be referred to as colloidal phosphorus throughout the rest of this discussion.) It seems likely that it is similar to "particles" (*6*) which form in seawater.

Four hypotheses can be advanced to account for the presence of the high-molecular-weight material (*7*): (i) it is the product of the direct adsorption of $[^{32}P]PO_4$ to colloids or small particles not retained by the Millipore filter; (ii) it is an artifact resulting from cell damage during filtration; (iii) it comprises small microorganisms which pass through the 0.45-μm filter; and (iv) it forms as a result of the complexing of an excretion product with colloids or small particles which pass through the Millipore filter.

I obtained evidence supporting hypothesis (iv) but not consistent with hypotheses (i), (ii), or (iii) by isolating the three filtrate components by gel filtration and adding each of them to a sample of freshly filtered lake water (no organisms present) (FLW). The results showed:

$$[^{32}P]PO_4 + FLW \rightarrow \text{no reaction} \quad (1)$$

$$XP + FLW \rightarrow \text{colloidal P} + [^{32}P]PO_4 \quad (2)$$

$$\text{Colloidal P} + FLW \rightarrow [^{32}P]PO_4 \quad (3)$$

On the basis of experiment 1, hypothesis (i) must be rejected; $[^{32}P]PO_4$ does not complex with anything. The other fractions appeared only when $[^{32}P]PO_4$ was added to unfiltered lake water; therefore, $[^{32}P]PO_4$ must have been taken up by organisms, which quickly excreted some of the isotope again as XP. This excreted material combined with nonlabeled colloids or small particles (experiment 2). When colloidal phosphorus was added to filtered lake water, part of it was converted to $[^{32}P]PO_4$ (experiment 3). On the basis of these results, I concluded that no high-molecular-weight material was excreted, only XP. This form combined with the colloid and in so doing removed a phosphate from a previous XP addition.

Other experiments revealed that, when lake water equilibrated with ^{32}P was filtered, XP disappeared, the amount of colloidal phosphorus remained the same, and the concentration of inorganic phosphate increased by an amount equal to the change in XP. The results of this experiment combined with the results of experiment 3 show that release of the $[^{32}P]$-PO_4 occurs as XP combines. Otherwise, the amount of colloidal phosphorus would decrease in this experiment too.

After the asymptote level was reached (Fig. 1) the distribution of isotope did not change significantly from 1 to 24 hours; therefore, a steady-state distribution was assumed and a typical 4-hour distribution of isotope was used to indicate the relative amounts of phosphorus in the four principal compartments (Fig. 2). Only 0.21 percent of the ^{32}P was inorganic phosphate. A typical value (*8*) for the total phosphorus in Heart Lake during this period was 44 μg/liter; therefore, if the nonlabeled phosphorus was distributed in the same way as the labeled phosphorus, then only 0.09 μg/liter was inorganic phosphate. This value is considerably below the sensitivity of the molybdate test for soluble reactive phosphate (*9*) and approximates Rigler's estimate based on the use of a bioassay technique (*1*).

Utilization experiments were conducted (*2*) whereby isolated fractions were added to freshly filtered lake water and the rate constants measured. The product of the concentration in each compartment and the rate constant is equal to the flux rate. The product of the concentration of the excreted XP form and k_3 approximates the product of the concentration of colloidal phosphorus and k_4. Therefore, the direct hydrolysis of XP to phosphate with a rate constant given as k_6 is not a significant pathway.

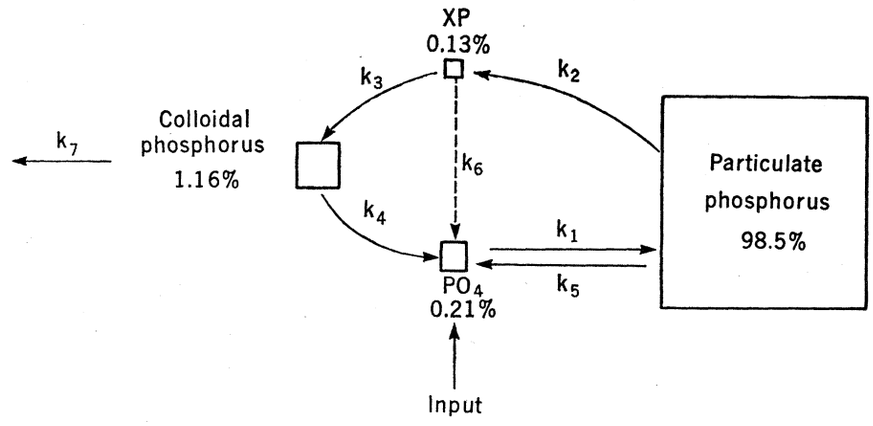

Fig. 2. Four principal compartments in the phosphorus model. An exchange mechanism exists between phosphate and the particulate fraction. Moreover, an organic phosphorus compound (*XP*) is excreted which combines with a colloidal substance. This step involves the release of phosphate from the colloid.

The uptake rate (k_1 multiplied by the phosphate concentration) must equal the rate of loss of isotope by the particulate fraction. This reaction can occur via pathways with rate constants k_2 and k_5. Mass balance calculations showed that k_5 must be about 100 times k_2. In other words, the loop via XP and colloidal phosphorus accounts for the deviation of the system from a simple two-compartment exchange model (*1*).

An input of phosphate is required in the model because, after extended periods of incubation (1 to 5 days), much of the colloidal phosphorus becomes biologically unavailable and is lost from the system. The rate constant for the loss of colloidal phosphorus (k_7) is small relative to the other rate constants but is important in overall lake metabolism. The relative importance of k_7 has been investigated in a series of in situ lake experiments, and these results will be presented elsewhere (*10*).

Fogg (*11*) has reported that there appear to be no published reports of freshwater algae excreting extracellular organic phosphorus compounds, but he thought it likely that such substances were normally excreted to some extent. The excretion of dissolved organic phosphorus by marine algae has been reported by many investigators (*12*), who claim that it occurs either during periods of rapid growth in excess phosphate with reutilization occurring during periods of low phosphate concentrations, or that it occurs during death and decay (*13*). However, on closer examination of the data (*12*), it appears that a consistent pattern emerges which contradicts their conclusions but is similar to my observations.

In my experiments organic phosphorus excretion took place near the period of maximum biomass when most of the phosphate had been utilized, not during the period of maximum growth. The excretion occurs throughout the summer period when phosphate would be in very short supply (that is, less than 0.09 μg/liter). The organic phosphorus excreted undoubtedly does not form from the death and decay of organisms (*13*), because the time (3 minutes) is far too short. Considerable retention of the colloidal phosphorus occurs during filtration, and, if no corrections are made, "utilization" is grossly overestimated. Polycondensation occurs as XP monomers are joined to the colloid, but in an aqueous medium it would not be surprising to find a substance such as phosphorus mediating the reaction. Long-term experiments in large polyethylene columns in a lake have shown that all of the X component of the XP molecule is permanently bound to colloidal material whereas most of the phosphorus of the XP molecule recycles (*10*).

The role of this colloidal phosphorus in lake metabolism remains obscure. It would seem that the excretion of XP by organisms is some sort of response to low phosphate or high biomass concentrations.

D. R. S. LEAN*
Department of Zoology,
University of Toronto,
Toronto, Ontario, Canada

References and Notes

1. F. H. Rigler, in *The Phosphorus Handbook*, A. M. Beeton, Ed. (Wiley, New York, 1972).
2. D. R. S. Lean and F. H. Rigler, in preparation.
3. D. S. Riggs, *The Mathematical Approach to Physiological Problems* (Williams & Wilkins, Baltimore, 1963), pp. 201–215.
4. E. T. Gjessing, *Nature* **208**, 1081 (1965).
5. E. H. McConkey, *Methods Enzymol.* **12** (part A), 630 (1967).
6. N. Ogura, *Limnol. Oceanogr.* **15**, 476 (1970); W. H. Sutcliffe, Jr., E. R. Baylor, D. W. Menzel, *Deep-Sea Res.* **10**, 233 (1963); P. J. Wangersky, *Amer. Sci.* **53**, 358 (1965).
7. Zooplankton do not contribute to this form. Similar kinetic data were obtained for lake water and lake water that had been passed through a 56-μm filter to remove major zooplankton.
8. F. H. Rigler, *Limnol. Oceanogr.* **9**, 511 (1964).
9. J. D. H. Strickland and T. R. Parsons, *Bull. Fish. Res. Board Can.* **167**, 1 (1968).
10. D. R. S. Lean and D. W. Schindler, in preparation.
11. G. E. Fogg, *Ergeb. Limnol.* **5**, 11 (1971).
12. N. J. Antia, C. D. McAllister, T. R. Parsons, K. Stephens, J. D. H. Strickland, *Limnol. Oceanogr.* **8**, 166 (196
13. R. E. Johannes, *ibid.* **9**, 224 (1964); E. J. Juenzler, *J. Phycol.* **8**, 7 (1970).
14. W. D. Watt and F. R. Hayes, *Limnol. Oceanogr.* **8**, 276 (1963).

I thank Dr. F. H. Rigler for his helpful suggestions during the experiments and Drs. F. H. Rigler, D. W. Schindler, and R. H. Peters for their helpful criticisms of the manuscript.

* Present address: Biogeochemical Limnology Subdivision, Lakes Research Division, Canada Centre for Inland Waters, Burlington, Ontario.

20 March 1972; revised 16 October 1972

Reaction of Hydrated Electrons with Ferricytochrome c

Abstract. *The reaction of ferricytochrome c with hydrated electrons produced at pH 6.8 by radiolysis with electron pulses lasting 50 to 1000 nanoseconds has a specific rate constant of* 5.5×10^{10} *liter mole*$^{-1}$ *sec*$^{-1}$ *for the formation of the primary adduct. By using appropriate wavelengths, another fast, consecutive process was demonstrated, with the pure first order rate constant* 1.0×10^{5} *sec*$^{-1}$. *Its characteristics agree with it being an intramolecular process within the enzyme. Approximately 50 percent of all electrons which add to ferricytochrome c end in forming ferrocytochrome c.*

The development of very fast pulse radiolysis techniques in aqueous solutions makes possible the quantitative production of simple radicals, such as hydrated electrons, e^-_{aq}; hydrogen atoms, H; and hydroxyl radicals, OH. These have only thermal energy and react in a specific and selective way with solutes. The reactions of cytochrome c (*1*) with e^-_{aq} or H (*2*) allow one to demonstrate the effects of one-electron equivalent reagents on oxidation reduction enzymes down to the diffusion-controlled time limit. The role of the protein in the electron equivalent transfer can also be demonstrated.

Using such methods, we studied the reactions of these radicals with the hydrolytic enzyme, ribonuclease, and showed that primary, very fast, nonselective addition to the protein was followed by consecutive first order intramolecular processes of radical transfer, affecting specifically and selectively the divalent sulfur and aromatic amino acid components in a linked process. For the hydrolytic enzyme, not designed for redox processes, there were irreversible chemical changes and enzyme inactivation (*3*).

Using spectrophotometric techniques, we studied the reaction of H atoms (produced by nanosecond pulse radiolysis) with Fe(III)-cytochrome c in aqueous solution at *p*H 2.5 and 6.5 (*4*). We found that H atoms add to the enzyme with the second order rate constant $k \cong 2 \times 10^{10}$ liter mole $^{-1}$ sec^{-1}. The primary addition was followed by three consecutive intramolecular processes, with first order rate constants of approximately 1×10^5, 2×10^4, and 2×10^3 sec^{-1}. The spectroscopic characteristics of the first two processes were consistent with the transfer of an electron equivalent through the protein to the Fe(III) moiety, to yield Fe(II). The slowest, third process could be attributed to configurational changes

We also reported briefly our results on the reaction of e^-_{aq} with Fe(III)-cytochrome c, for which we obtained $k \cong 4 \times 10^{10}$ liter mole^{-1} sec^{-1}. Hydrogen atoms do not absorb energy in the spectroscopic range of cytochrome c (370 to 650 nm). Above about 500 nm, e^-_{aq} absorbs sufficiently to complicate observations on the transition Fe(III) → Fe(II). Accordingly, processes involving e^-_{aq} are somewhat harder to elucidate than those involving H.

Land and Swallow (*5*) studied the reaction of e^-_{aq} with Fe(III)-cytochrome c by using pulses of approximately 200 to 400 rads and cytochrome c concentrations of 4×10^{-6} to $3 \times 10^{-5}M$. At a *p*H of about 7 they found $k \cong 2 \times 10^{10}$ liter mole^{-1} sec^{-1}. In their system, 0.1*M* formate buffer was present, and they corrected for its reaction with the radicals produced by irradiation. Their results at *p*H 7 were interpreted as showing that within 150 nsec Fe(III) was converted to Fe(II), the rate of reduction being equal to the rate of addition.

Pecht and Faraggi (*6*) reported similar experiments, with 100-nsec pulses (approximately 400 to 500 rads) at cytochrome c concentrations of 5×10^{-7} to $3 \times 10^{-6}M$, but they found $k \cong 1.5 \times 10^{11}$ liter mole^{-1} sec^{-1}. The interpretation of their results is complicated by the fact that at the low concentrations and relatively high dose employed, there are more e^-_{aq} available than Fe(III)-cytochrome c. They assumed that at the very high rate constants they calculated, e^-_{aq} reacts directly with the Fe(III) moiety.

For the experiments reported here we used deaerated aqueous solutions of Fe(III)-cytochrome c (Sigma type IV, 95

Table 1. Apparent first order rate constants (k_{obs}, in sec^{-1}) and calculated second order rate constants (k_1, in liter mole^{-1} sec^{-1}) for the reaction between ferricytochrome c and e^-_{aq} at *p*H ~6.8.

Cytochrome c $\times 10^{-5}M$	650 nm		580 nm		535 nm		520 nm		510 nm		370 nm	
	$k_{obs} \times 10^5$	$k_1 \times 10^{10}$	$k_{obs} \times 10^5$	$k_1 \times 10^{10}$	$k_{obs} \times 10^5$	$k_1 \times 10^{10}$	$k_{obs} \times 10^5$	$k_1 \times 10^{10}$	$k_{obs} \times 10^5$	$k_1 \times 10^{10}$	$k_{obs} \times 10^5$	$k_1 \times 10^{10}$
5	27 ± 3	5.4 ± 0.6	27 ± 3	5.4 ± 0.6	30	6.0	30	6.0				
1	6.2 ±1.7	6.2 ± 1.7	5.5 ± 1.5	5.5 ± 1.5	6.8 ± 1.7	6.8 ± 1.7	5.1 ± 1.4	5.1 ± 1.4	5.3 ± 0.5	5.3 ± 0.5	4.2 ± 0.2	4.2 ± 0.2
0.5	2.8 ± 1.7	5.6 ± 0.8	3.0 ± 0.7	6.0 ± 1.4							2.5 ± 0.3	5.0 ± 0.6
0.25	2.4 ± 0.4	[10]	2.5 ± 0.8	[10]	2.2 ± 0.3	[9]	2.3 ± 0.3	[9]			1.2 ± 0.2	[4.8]