PELAGIC PHOSPHORUS METABOLISM IN ANTARCTIC WATERS[1]

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ABSTRACT

Eleven large quantitative samples of the particulate material in Antarctic and subantarctic surface waters were obtained with a continuous flow system. Each sample was divided into those particles which were retained by a No. 20 plankton net with a pore size of about 30 μ (class I) and those which passed through the net but were retained by a Sharples Supercentrifuge (class II). Total phosphorus and organic nitrogen were determined on aliquots of class I and II samples, and the remaining material was chemically fractionated into acid-soluble organic phosphorus, phospholipids, orthophosphate, oligopolyanions, and ribonucleic acid-polyphosphates.

About two-thirds of the nonparticulate phosphorus was orthophosphate. From 3 to 7.5% of total phosphorus was particulate. Over half of the particulate phosphorus was in class II; ^{32}P-phosphate experiments indicated that these particles incorporated phosphate at least six times as rapidly as class I particles. In class II particles orthophosphate and RNA-polyphosphate constitute 80 to 85% of the total phosphorus; in class I the phospholipids are also a large fraction. RNA-polyphosphate was the most rapidly labeled fraction from the class II particles.

INTRODUCTION

Data have been gathered concerning the levels of phosphorus occurring in both water and plankton at the surface and at various depths at many locations and times of year. At the same time, many laboratory studies have thrown light on the identity and quantity of various phosphorus compounds in plants and animals. These have demonstrated that the bulk of the phosphorus is in the form of polyphosphate and orthophosphate in bacteria (Sall, Mudd, and Takagi 1959; Zaetseva, Khmel, and Belozerskii 1961) and in fungi (Belozerskii and Kulaev 1957; Kulaev and Belozerskii 1957). Studies such as that of Zaetseva, Belozerskii, and Frolova (1960) on *Azotobacter* have shown that the polyphosphate fraction is rapidly metabolized. These conclusions have been extended to pure cultures of algae (Nihei 1957; Schmidt and King 1961; Correll and Tolbert 1962 and 1964; Overbeck 1962; Schweiger and Bremer 1960). In contrast, higher plants (Miyachi 1961; Tewari and Singh 1964), insects (Heller, Karpiak, and Zubikowa 1950), and vertebrates (Lynn and Brown 1963) have only small amounts of polyphosphates. Under a number of conditions, such as certain stages of cell development or some types of growth inhibition, microorganisms accumulate polyphosphate.

Such flexibility in phosphorus metabolism helps explain the large changes in total phosphorus per cell observed by Lund (1950) in *Asterionella* blooms, the 17-fold shifts in the P : N ratio of *Chlorella* induced by nutritional deficiency (Ketchum and Redfield 1949) and the 33-fold changes in phosphorus per *Phaeodactylum* cell induced by changes in the level of phosphate in the culture medium (Kuenzler and Ketchum 1962).

In this study, an attempt has been made to obtain quantitative pelagic samples adequate to determine the distribution of phosphorus among five classes of phosphorus compounds. In addition, an effort has been made to obtain data concerning the relative rates of phosphorus incorporation into these classes of compounds under conditions approximating those the organisms encounter in nature. An area rich in phosphate (Reid 1962) was chosen to investigate one extreme of the spectrum of phosphorus conditions.

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METHODS

Samples were collected from the *USNS Eltanin* between South America and 115° W long and from 40° S lat to 70° S lat (Fig. 1). Seawater was taken from a depth of 6 m by means of a pipe that penetrated the hull amidships and passed in a continuous flow system, first through a silk bolting No. 20 plankton net with a pore size of about 30 μ (class I particles), and then through a Sharples Supercentrifuge (air turbine) operating at 30–35,000 rpm (11,000 × g) to obtain class II particles. Aliquots of the effluent seawater were also collected. Class I and II particles were transferred to 50-ml centrifuge tubes at the conclusion of each 2-hr run and centrifuged 10 min at 1,500 rpm and 0C (International, model PR-2 refrigerated centrifuge). The pellets and water samples were frozen and stored at temperatures lower than −20C until analyzed. The entire sampling procedure was carried out below waterline so pumping was unnecessary. A flow rate of approximately 1.5 liters/min was obtained and frequent checks were made of the flow rate in order to calculate the volume of seawater processed. All collecting was done during daylight. In laboratory tests this collecting system was adequate to clarify cultures of *Escherichia coli* or *Phaeodactylum tricornutum*.

Sample I was obtained by melting several blocks of sea ice collected at the location noted in Fig. 1. This ice was brown and heavily colonized with pennate diatoms. Without passing it through a plankton net and before warming it above 2C, 50 liters of melt were centrifuged with the Sharples centrifuge.

Pellets were pooled from geographically adjacent runs to obtain sufficiently large samples for fractionation. Phosphorus extractions and fractionations were carried out by thawing frozen pellets of class I or class II particles in 50 ml of 5% trichloroacetic acid (TCA) at 2C and grinding them in a glass homogenizer. At this stage, aliquots were taken for organic nitrogen and total phosphorus determinations. The residue from the first TCA extraction was reextracted in the same way with 50 ml of 5% TCA at 2C. The combined TCA extracts were mixed with 0.6 volume concentrated NH_4OH and 0.1 volume 15% $MgCl_2$. After 2 hr at 0C, the precipitate was separated, leaving the organic phosphate ester fraction in solution. The magnesium hydroxide precipitate was dissolved by adding 6 N HCl to a *p*H of 1. Then 4 volumes of cold 95% ethanol were added and the solution was kept at 0C for 2 hr. The precipitate, which was the oligopolyanion fraction, was separated. The supernatant was the orthophosphate fraction. The residue from the TCA extractions was extracted by grinding with 50 ml of ethanol-ether (3/1) in a glass homogenizer. The residue from the first ethanol-ether extraction was reextracted in the same way and the combined ethanol-ether extracts constituted the phospholipid fraction. The residue was the RNA-polyphosphate fraction. In each step the precipitate was separated by centrifuging 15 min at 10,000 × g and 0C in an International,

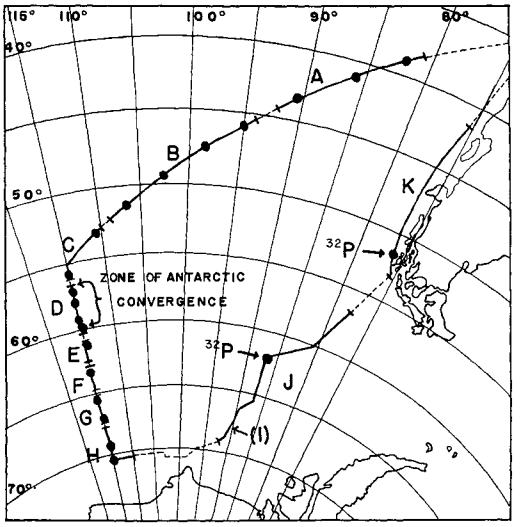

FIG. 1. Sampling locations on Cruise 11, *USNS Eltanin* in the U.S. Antarctic Research Program (20 Dec 1963–17 Feb 1964). Lettered bars along the route represent areas where large quantitative samples of particulate material were collected; solid circles represent points at which water samples for nutrient analysis were taken. The letter I denotes the location at which a pack ice sample was taken. ^{32}P-incorporation experiments were done at the locations marked.

TABLE 1. *Major nutrients in seawater*

Sample	Seawater processed (m^3)	μg at./liter (average)			
		total-P	ortho-P	nitrate	silicate
A	1.8	1.1	0.7	1.2	0.1
B	2.4	1.4	0.9	1.5	0.9
C	1.6	1.4	0.9	1.3	0.8
D	2.2	1.6	1.1	1.8	2.8
E	1.6	1.6	1.1	2.4	3.5
F	1.4	2.4	1.6	3.2	25
G	1.8	1.8	1.3	2.2	27
H	1.4	1.9	1.3	2.0	48
J	1.8	1.3	1.4	4.7	5.2
K	2.6	1.6	0.8	0.8	4.0

model HR-1, refrigerated centrifuge. Particulate total-phosphorus and orthophosphate were determined by the methods of King (1932). Organic nitrogen in particulate samples was determined by the micro-Kjeldahl procedure (Belcher and Godbert 1945). Before digestion, the nitrogen samples were treated with 0.2 volume 12 N NaOH at 100C for several minutes while an air jet was passed over them to remove any ammonia present.

Total-phosphorus, orthophosphate, and nitrate in centrifuge effluent samples were determined using the methods described by Strickland and Parsons (1960). Reactive silicate was determined by the direct molybdate method (Chow and Robinson 1953).

In order to study phosphate incorporation, the combined class I and class II particulate material from a run was transferred directly from the collecting apparatus to a 22-liter roundbottom glass flask. Seawater of the prevailing surface temperature was used for the transfer and to dilute the sample to 20 liters. The flask was then suspended in a tank of seawater that was open to the sky on the upper deck. The tank was maintained at ± 1C of the surface water temperature at the sampling site. The sample was allowed to equilibrate overnight. Experiment J was done at 5C, while K was done at 11C. At dawn, 5 mc of carrier-free ^{32}P-orthophosphoric acid were added. Ten liters were harvested at 2 hr and the rest at 12 hr. Samples were run through the ordinary harvest system, and care was taken always to use ^{32}P-containing overflow from

TABLE 2. *Total phosphorus and organic nitrogen/m^3 in particles of class I and II*

Sample	mg total phosphorus/m^3		mg organic N/mg T-P	
	I	II	I	II
A	0.2	0.8	—	—
B	0.7	1.0	—	—
C	0.8	1.4	—	—
D	1.3	1.6	53	6.7
E	1.4	2.1	70	23
F	1.1	2.4	46	8.0
G	1.9	2.0	78	57
H	0.7	2.4	368	24
J	1.3	1.6	7.7	6.7
K	1.1	2.0	9.5	5.7
I*	29.6		6.4	
PD† (Normal)			4.9	
PD† (P-deficient)			7.5	
PD† (NO^-_3-deficient)			0.56	

* I was pack ice sample.
† PD = *Phaeodactylum tricornutum* cells.

the centrifuge when transferring and centrifuging the particles. Two incorporation experiments were conducted (Fig. 1). All radioactive samples were counted with a Nuclear-Chicago, model C-110B, thin window, gas-flow counter system. In some cases orthophosphate was counted by first developing the molybdic acid blue complex, then extracting it with isobutanol. To fractionate the radioactive samples, carrier samples of cold material collected in nearby areas were homogenized with twice the normal volume of cold trichloroacetic acid. The homogenized samples were then divided into two equal parts, one for the 2-hr incorporation sample, the other for the 12-hr sample. In this manner the radioactivities of the 2-hr and 12-hr samples were equally diluted with carrier.

Chlorella pyrenoidosa cultures were grown as described by Correll and Tolbert (1962) and *Phaeodactylum tricornutum* cultures were grown as described by Guillard and Ryther (1962). For comparison, these cells were also fractionated. In addition, *Phaeodactylum tricornutum* was serially transferred into artificial seawater media (Droop 1955) lacking either phosphate or nitrate until final cell densities were severely limited. The nitrate- or phosphate-deprived cells were then washed three times with 3% sodium chloride solu-

tion and aliquots were analyzed for total phosphorus and organic nitrogen.

RESULTS

Average water analysis values within sample areas are given in Table 1. Total phosphorus was always over 1 μg-at./liter, and about one-third was in some form other than orthophosphate. Nitrate was always easily detectable, but reactive silicate was very low in area A, and less than 1 μg-at./liter in areas B and C. South of the Antarctic Convergence, the values of orthophosphate and nitrate were generally higher, but the reactive silicate, in agreement with the work of Clowes (1938), was much higher.

Table 2 shows that particulate phosphorus varied from one to almost 4 mg/m^3. Class II particles contained about 50% more phosphorus than class I particles. Particulate phosphorus varied from 3% of dissolved phosphorus in sample A to almost 7.5% in sample G. The sea-ice diatom community, sample I, contained about 10 times as much particulate phosphorus per volume as surface waters in the same vicinity. Table 2 also contains data obtained with laboratory cultures of *Phaeodactylum tricornutum* under "normal," nitrate-deficient, and phosphate-deficient conditions. The N : P ratio in these cultures varied from 7.5 in phosphate-deficient conditions to 4.9 in "normal" medium to 0.56 in nitrate-deficient conditions. The ice community sample had a ratio of 6.4 and fits within the expected range. However, the N : P ratios of the other samples range from 5.7 to 368. In all cases, class II particles had a lower ratio than class I particles.

Phosphorus fractionations of samples A through I and of laboratory algal cultures (Table 3) show that class I particles, which contained almost all of the zooplankton as well as variable amounts of phytoplankton, had a much larger proportion (13 to 29%) of phospholipid than class II particles. Class II particles, however, contained much higher levels (27 to 57%) of RNA-polyphosphate than class I particles. Both classes of particles contained more orthophos-

TABLE 3. *Per cent distribution of particulate phosphorus*

Fraction	A-I	A-II	B-I	B-II	C-I	C-II	D-I	D-II	E-I	E-II	F-I	F-II	G-I	G-II	H-I	H-II	I (I & II)	PD*	CH†
acid-soluble organic-P	11	4	2	4	4	6	8	8	15	9	9	8	12	6	8	7	3	6	7
P-lipid	25	5	16	4	16	4	29	7	24	6	27	6	13	4	15	4	7	7	3
ortho-P	36	32	53	31	54	35	44	41	34	45	38	53	42	45	45	59	37	12	23
oligopolyanions	6	6	2	4	3	6	4	6	4	5	9	3	5	11	6	3	6	1	1
RNA-poly-P	22	53	27	57	23	49	15	38	23	35	17	30	28	34	26	27	47	74	66

* *Phaeodactylum tricornutum.*
† *Chlorella pyrenoidosa.*

TABLE 4. *^{32}P-incorporation into particles*

Fraction	(Sample J)*						(Sample K)†					
	Class I			Class II			Class I			Class II		
	% T-P	Specific‡ activity		% T-P	Specific‡ activity		% T-P	Specific‡ activity		% T-P	Specific‡ activity	
		2 hr	12 hr		2 hr	12 hr		2 hr	12 hr		2 hr	12 hr
acid-soluble organic-P	10	8	74	5	84	820	9	30	400	7	800	2,300
P-lipid	11	17	250	8	450	2,200	19	24	260	4	900	6,400
ortho-P	45	170	470	47	410	1,100	40	330	910	40	1,600	1,800
oligopolyanions	4	26	230	1	920	3,600	3	130	710	8	520	900
RNA-poly-P	30	29	570	39	870	2,800	29	160	1,300	41	1,700	4,700

* Specific activity of ortho-P in medium $= 5 \times 10^5$ (1/9) $= 6 \times 10^4$.
† Specific activity of ortho-P in medium $= 1.2 \times 10^6$ (1/13) $= 9 \times 10^4$.
‡ Counts min^{-1} (μg P)$^{-1}$.

phate and oligopolyanions than were found in healthy laboratory cultures, indicating the presence of significant levels of detritus and unhealthy plankton. This type of enzymatic degradation can be illustrated in the laboratory by storing *Phaeodactylum* cells at –1C. Within 2 days there is a shift from 74 to 28% RNA-polyphosphate and from 12 to 64% orthophosphate.

The results of the fractionation and counting of samples J and K (^{32}P-labeled material plus carrier material) are presented in Table 4. The specific activities are in all cases those obtained at the time of fractionation. They illustrate differences in relative rates of incorporation of ^{32}P-phosphate. All counts were well above background and in most cases at least 20 times that of background. At 12 hr the highest specific activity (sample K, class II phospholipids) was about 6.5% of the specific activity of the orthophosphate in the medium if the data are corrected for carrier dilution. Class II particles incorporated ^{32}P-phosphate at least six times as rapidly as class I and showed a different pattern of incorporation into the various phosphorus fractions. RNA-polyphosphate was labeled most rapidly by class II particles (pool size × specific activity), but phospholipids were also rapidly labeled and reached a high specific activity. The more abundant phospholipids in class I particles were labeled at a slower rate. Here it is evident that orthophosphate had the highest specific activity at 2 hr, and the RNA-polyphosphate fraction had the highest specific activity at 12 hr.

DISCUSSION

The data from these experiments are in general agreement with previous fieldwork with plankton and laboratory studies of phosphorus metabolism. The experiments were designed to measure phosphorus metabolism in the plankton community with the realization that only a first order approximation might be obtained with the methods used. No effort was made to measure ammonia-nitrogen in the water or to identify the organic phosphorus compounds in the water.

The phosphorus fractionations used in the analysis of particulate samples were purposely designed to be flexible enough to be effective regardless of the biological materials present. In all cases, the orthophosphate fractions were verified to be over 90% orthophosphate through developing the phosphomolybdic acid blue complex and extracting it with isobutanol. The RNA-polyphosphate fraction also contained DNA and phosphoproteins, but they always constituted less than 10% of the fraction. Oligopolyanions included primarily oligopolynucleotides and oligopolyphosphates.

Since animal tissues contain more phospholipids than algae do, but only traces of polyphosphate, class I particles were higher in phospholipids and lower in RNA-polyphosphate than class II particles. The

ratio of phytoplankton to zooplankton in class I particles was noticeably higher in and just south of the Antarctic Convergence due to filamentous diatoms retained by the net. Microscopic examination of class II particles showed them to be predominantly diatoms. Consequently, their composition more closely approached that of laboratory algal cultures.

The very high N : P ratios (Table 2) were unexpected. The explanation may be that the phosphorus components are more rapidly degraded and leached from dead plankton, eventually leaving resistant nitrogenous detritus such as chitin and cell wall materials.

The ^{32}P-incorporation experiments were intended to obtain order-of-magnitude data since they were conducted within a glass flask of 20 liters volume. It was necessary to subject the plankton to possible damage from centrifugation and handling in concentrating from 200 liters to 20, although care was taken to avoid temperature or salinity changes. Treatment of this kind causes little damage to laboratory cultures of marine diatoms. Microscopic examination of class II particles revealed that diatoms were the principal group and no damage was apparent. Examination of the data shows that some of the plankton survived and metabolized. Class II particles were labeled much more rapidly, as expected, since the zooplankton of class I feed on class II particles. Also, class II particles labeled RNA-polyphosphate most rapidly, as is typical of microbiological cultures in the laboratory (Kulaev and Belozerskii 1957; Langen and Liss 1958), while class I particles labeled orthophosphate most rapidly. This is probably because the filter feeders degrade phosphates in their food to orthophosphate before assimilating it. Furthermore, direct phosphate uptake by class I particles might be expected to be slower due to their lower specific surface area. By 12 hr, many fractions had higher specific activity than particulate orthophosphate, indicating a sizeable, metabolically inert, pool of particulate orthophosphate. This is probably due to a number of factors, one being the presence of detritus, which is also indicated by the high orthophosphate levels in all of the particulate samples.

The actual rate of ^{32}P-incorporation for the first 2 hr in sample J (class II polyphosphates) was 0.7% per hr. In sample K (class II polyphosphates) this rate was 0.9% per hr. These rates are the best estimate of actual living diatom synthetic rates that we can obtain from the data and correspond to about 10% for a 12-hr day.

No bacterial counts were made during the ^{32}P-incorporation experiments. However, all glassware was washed with sulfuric acid and rinsed with distilled water before use. The size and shape of the incubation flask reduced the glass surface area to a minimum. The centrifuge and plankton net were washed and dried between each 2-hr collecting period. Despite these precautions, bacteria were undoubtedly present and may have been present in unusual numbers.

Major nutrients for diatom growth, particularly silicate, were more abundant at the convergence (Fig. 1, sample D) and south of that area (Table 1). A much greater concentration of biomass was observed in sample D than in samples A, B, and C. It was expected that the total phosphorus of the particles in these samples would reflect these facts. However, class II particles showed only a three-fold variation in total phosphorus/m^3. Class II particles of samples A, B, and C did show an increase in total phosphorus with latitude as expected, but no dramatic increase occurred in sample D. Class I particles showed a somewhat greater variation in total phosphorus/m^3. One possible explanation for the lack of dramatic change in class II particles phosphorus may be that phytoplankton that is actively growing doesn't store as much phosphorus per cell as it does when growth is retarded (Wintermans 1955). This explanation is supported by the distribution of phosphorus found in class II particle fractionations (Table 3). Samples A, B, and C contained much higher percentages of RNA-polyphosphate than did samples D through H. These compounds are stored under conditions that limit growth

without limiting available phosphate or metabolic energy. Thus, the phytoplankton north of the convergence probably contained more phosphorus per cell than those south of it.

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