Vitamin levels in the Gulf of Maine and ecological significance of vitamin B_{12} there

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ABSTRACT

Bioassays were used to determine the concentration of vitamin B_{12}, cobamides (B_{12} + analogs), thiamin, and biotin in Gulf of Maine water samples from winter, the spring bloom, and summer. Vitamin B_{12} had a range of 0.1 to 1.9 ng l^{-1}, cobamides were 0.1 to 4 ng l^{-1} indicating that analog concentration equals or exceeds that of vitamin B_{12} except during the spring bloom. Binding factor was present except during the spring bloom. Centric diatoms from the spring bloom lack an absolute requirement for B_{12} but are stimulated by it (shorter lag phase or increased growth rate). B_{12} decreases during the spring bloom and its presence in high concentration in phytoplankton particulate material suggests that the bloom diatoms utilize the external supply of vitamin B_{12} and benefit from its stimulatory effect. Thiamin was 10-45 ng l^{-1} and biotin was 1-11 ng l^{-1}; neither of these two vitamins seems to have a controlling influence on phytoplankton in the Gulf of Maine.

1. Introduction

Vitamins have been proposed as chemical constituents which can have an ecological role, such as affecting phytoplankton abundance, species composition, or growth rate (Daisley, 1957; Provasoli, 1958, 1963; Ford, 1958; Guillard and Cassie, 1963; Riley, 1966; Swift and Taylor, 1974, Swift, 1980). There are also less direct aspects to consider in addition to the effect of a particular vitamin concentration on growth rate or cell number of a given species: (1) Some clones can utilize vitamin B_{12} analogs to meet a vitamin B_{12} requirement (Guillard, 1968; Swift and Guillard, 1977). (2) Usually a vitamin response is an absolute requirement, but some clones show a stimulatory response to a vitamin such as that of Gulf of Maine centric diatoms to B_{12} (Swift and Guillard, 1978) or of some green algae to thiamin (Turner, 1979). (3) Algae can take up vitamin B_{12} in excess very rapidly (Droop, 1968). (4) Many clones produce an excess of the vitamins for which they have no absolute requirement and thus act as sources of vitamin to the water column or other species (Carlucci and Bowes, 1970a, 1970b; Swift and Guillard, 1978). (5) Many algae produce a binding protein which makes B_{12} unavailable for uptake (Droop, 1968; Pintner and Altmyer, 1973, 1979).

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Field and laboratory studies have addressed the relationship between vitamins and phytoplankton in several geographical regions. Menzel and Spaeth (1962) found vitamin B_{12} concentrations to be quite low in the Sargasso Sea in samples from above 100m. The range was about 0.01 to 0.1 ng l^{-1}; sometimes none was detectable. Concentrations increase during winter, reaching the high value in April during the small spring phytoplankton bloom dominated by centric diatoms (Hulburt, Ryther and Guillard, 1960). Vitamin B_{12} concentrations and primary production drop rapidly after this and the dominant phytoplankter becomes *Coccolithus huxleyi*, which requires only thiamin. A number of the diatom species in culture isolated from the Sargasso Sea require vitamin B_{12} (Guillard, 1968; Menzel and Spaeth, 1962), but vitamin B_{12} enrichment of water samples does not increase C^{14} uptake (Menzel and Ryther, 1961). The Sargasso Sea is low in nutrients; iron and silicate, which did sometimes stimulate uptake (Ryther and Guillard, 1959), in addition to vitamin B_{12} are considered to be low or limiting. Effects of vitamin B_{12} there probably occur as part of multiple nutrient limitation.

In Long Island Sound (Vishniac and Riley, 1961) maximum vitamin B_{12} concentrations of 16 ng l^{-1} are reached in January following fall and winter cooling and mixing. Decrease in B_{12} concentration occurs while *Skeletonema costatum* is undergoing rapid growth and dominates the winter-spring bloom of centric diatoms. Concentrations remain at 4 to 5 ng l^{-1} between February and April. Concentrations then increase through summer and fall, interrupted by a rapid drop during September which coincides with a smaller autumn bloom. Propp (1970) describes the phytoplankton cycle and some vitamin B_{12} measurements in Dal'niye Zelentsy Inlet in the Barents Sea, where there are two spring diatom blooms. During the first one, which *S. costatum* dominated, B_{12} was undetectable at one time although maximum yearly values were about 2 ng l^{-1} prior to spring blooms. During the second bloom, *S. costatum* and *Leptocylindrus danicus* alternated dominance. M. Fiala (Banyuls, France, personal communication) found a vitamin B_{12} requirement in *L. danicus* of Mediterranean origin. Thus *S. costatum* and *L. danicus* may be in competition for the B_{12} present. The B_{12} requirement of *S. costatum* is well documented for isolates of various origin (Droop, 1955; Riley, 1966; Guillard and Cassie, 1963; Carlucci and Bowes, 1970 a, b; Turner, 1979). *S. costatum* blooms usually begin when B_{12} concentration is far in excess of the value of the half-saturation constant for growth rate, 0.32 ng l^{-1} as determined for chemostat culture (Droop, 1970). Persistence of this species while vitamin B_{12} concentration is low or undetectable is dependent on a source of B_{12} (probably from mixing in the case of winter-spring blooms) after the cells have used up excess internal stores.

Carlucci (1970) found no dependence of phytoplankton production on vitamin concentrations (B_{12}, thiamin and biotin) in the coastal Pacific near La Jolla, California. However he suggests that there may be effects which are important for particular species and describes an occasion in which B_{12} concentration dropped suddenly during a week when *Gonyaulax polyedra* increased rapidly.

Cattell (1969) studied the annual cycle of B_{12} and dinoflagellates in the Strait of Georgia, B. C. Vitamin B_{12} concentration increased in the fall with breakdown of stratification. It decreased during the spring bloom and increased after the bloom. Another peak was associated with summer river runoff. Dinoflagellate population was statistically correlated with vitamin B_{12} concentration in early spring. In summer there was correlation with nitrogen. At other times no significant correlations were found. Diatoms, which initiate the spring bloom, were not enumerated.

The Gulf of Maine phytoplankton is characterized by large populations of centric diatoms (Bigelow, 1926; Bigelow, Lillick and Sears, 1940) with spring cell density commonly tens to hundreds of thousands per liter throughout the offshore waters. *Thalassiosira nordenskioeldii* begins the bloom and is the dominant species. After it has become well established, other *Thalassiosira* species and numerous species of *Chaetoceros* grow but do not attain the density reached by *T. nordenskioeldii.* In summer, autumn, and winter, dinoflagellates are dominant, especially species of *Peridinium* and *Ceratium.* Diatoms are dominant only in coastal area (inside the 100m depth contours), where *S. costatum* is one of the dominant species. The diatom *Detonula confervacea* and the coccolithophore *Coccolithus huxleyi* are also present in the summer plankton of the Gulf. Occasionally in summer or winter, oceanic water from east of the Gulf will carry in tropical and subtropical species, noticeable only if the plankton is otherwise sparse (Bigelow, 1926, Ketchum, 1968). The importance of nannoplankters is not known, as most studies used nets, preservatives or centrifuging techniques which discriminate against them.

Prior to the spring bloom, vertical mixing results in high nutrient content, and the nearly uniform upper 100m layer. The few cells present are not able to increase rapidly until slight stabilization of the surface layer occurs, according to critical depth concept (Gran and Braarud, 1935; Riley, 1942; Sverdrup, 1953; Ketchum, 1967, 1968). Most of the diatoms in the spring flowering can form resting spores (Cupp, 1943; Heimdal, 1974; Durbin, 1978). The spores of *T. nordenskioeldii* form and survive only at low temperature (Durbin, 1978). In the Gulf of Maine they ought to be able to survive over summer at the bottom in deeper water (100-200m); however the bloom begins in coastal areas and over offshore banks and spreads later to the deeper central Gulf.

Waters south and west of this area such as Vineyard Sound (Lillick, 1937; Fish, 1925), Block Island Sound (Riley, 1952), Long Island Sound (Riley and Conover, 1967) and Narragansett Bay (Pratt, 1959; Smayda, 1973) are characterized by a winter population of diatoms which begins to bloom during December or January while winter cooling continues. Several diatom pulses may occur before summer.

The goal of this study was to investigate the ecological role of vitamin B_{12} by measuring vitamin concentrations in the water at different times and by studying the vitamin responses of phytoplankton species isolated freshly from the same water, an approach suggested by Provasoli (1958). The Gulf of Maine was chosen because the spring bloom there occurs after the period of nutrient replenishment (from

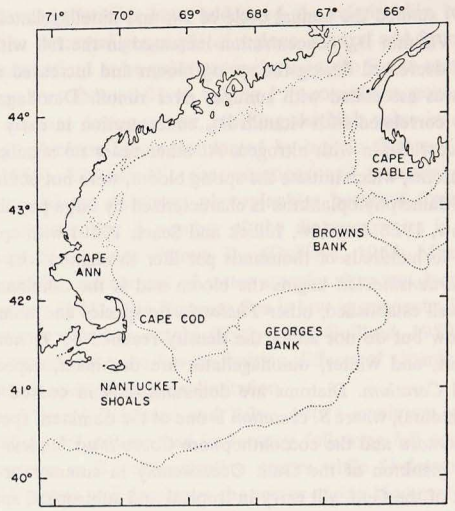

Figure 1. The Gulf of Maine with offshore contour of 100m shown.

deeper water and sediments). Thus changes in vitamin concentration should reflect biological activity rather than primarily mixing. Characteristic diatoms isolated from the spring bloom were found to be autotrophic for vitamin B_{12}, but they behave as auxotrophs in the laboratory by growing faster or with a shorter lag phase if B_{12} is present (Swift and Guillard, 1978), a response unlike almost all other centric diatoms studied so far (Provasoli and Carlucci, 1974). The results of various vitamin assays, and in particular those from water samples during the diatom bloom, will be examined in order to determine the role of vitamins in the Gulf of Maine.

2. Materials and method

Gulf of Maine sampling. Figures 1 and 2 show locations of stations at which samples were taken for vitamin assay. Table 1 gives additional details. Temperatures are shown as a guide to season and stratification.

The three stations of R/V *Chain* cruise 80 were made while following a drogue placed at a depth of 10m.

Water samples collected on R/V *Gosnold* cruise 130, August 1968, were lost during storage in a freezer room at Woods Hole Oceanographic Institution. Samples from *Gosnold* cruise 168 were taken subsequently to provide summer samples.

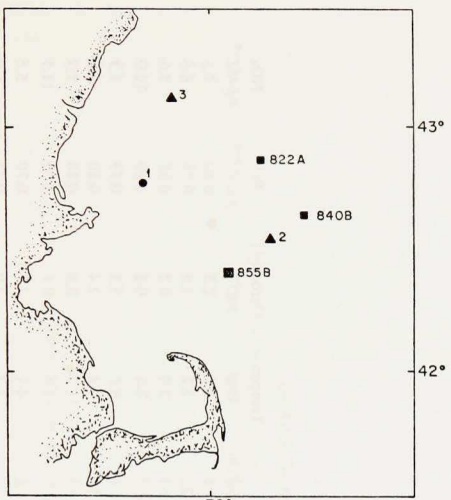

Figure 2. Location of stations.

Sea water samples were taken with van Dorn or Niskin samplers. Samples were kept chilled until filtered by vacuum through 0.45 μ membrane filters (Millipore Corp.) in glass apparatus. They were then frozen in polyethylene bottles until assayed. Filtration of samples from R/V *Gosnold* cruise 168 was slightly different. These were filtered under pressure into acid-washed polyethylene containers which had been rinsed with filtered sea water.

Additional whole water samples were taken at 1m and 20m at the three stations on the spring cruise, R/V *Chain* 80. These were autoclaved on shipboard to break open algal cells and liberate particulate or bound vitamin B_{12}. The samples were then transferred to plastic bottles for freezing and storage.

Vitamin analysis: general aspects. Samples to be assayed were thawed, filtered, diluted to 90% strength with fresh glass distilled water, fortified with nutrients other than the vitamin under assay, and diluted if required (with vitamin-free Gulf of Maine sea water medium). Procedures follow those described by Swift and Guillard (1977).

Vitamin B_{12} and cobamide concentration. Assay for vitamin B_{12} was performed with the diatom *Thalassiosira pseudonana* clone 3H. This organism responds significantly only to vitamin B_{12} (cyanocobalamin or 5,6 dimethylbenzimidazolyl coba-

Table 1. Location, temperature and nutrients at stations sampled for vitamin assay.

Cruise	Station & Date	Location	Depth	Depth, m	Temperature	Chlorophyll $\mu g\ l^{-1}$	PO_4 μg at l^{-1}	NO_3 μg at l^{-1}
Chain 80:	822A	42°52.5′N	250m	1	2.9	2.3	0.80	6.5
(spring bloom)	4/4/68	69°51.0′W		20	2.7	1.6	0.75	6.6
				50	2.0	0.2	0.95	7.6
				100	2.8	0.1	1.00	10.0
	840B	42°43.8′N	250m	1	3.6	3.3	0.75	6.3
	4/10/68	69°42.0′W		20	3.1	2.4	0.80	7.6
				50	2.9	0.5	0.80	7.7
				100	3.4	0.1	1.00	11.1
	855B	42°40.5′N	210m	1	4.1	4.3	0.70	5.6
	4/15/68	69°54.8′W		20	3.7	3.0	0.75	6.0
				50	3.1	0.5	0.85	8.1
				100	3.2	0.1	0.95	10.0
Gosnold 168:	2	42°38.4′N	275m	1	19			
(summer)	8/13/70	69°36.0′W		20	17			
				50	3			
				100	—			

	3	42°05.4′N	190m	1	21			
	8/13/70	70°11.2′W		20	12			
				50	8			
				100	—			
Rorqual 169:	1	42°42′N	100m	1	3.9			
(winter)	1/23/69	70°29′W		20	4.2			
				50	5.2			
				100	5.3			
		Nutrient data from other cruises to same location:						
Atlantis II 26:	Diurnal I	42°53.0′N	258m	1	16	3.0	0.15	0.3
(late summer)[a]	9/15/66	69°53.0′W		20	13.6	3.2	0.20	0.2
				50	6	0.7	0.80	8.0
				100	4.3	0.2	0.95	11.6
Chain 65:	Diurnal I	42°52.7′N	233m	1	2.5	1.3	0.70	6.1
(pre-bloom)[b]	3/28/67	69°53.2′W		20	2.5	1.8	0.75	6.1
				50	2.5	1.2	0.70	5.9
				100	3.2	0.8	0.75	7.7

[a] (Ketchum, 1967) [b] (Ketchum, 1968)

mide cyanide) and to its chemical form hydroxocobalamin which occurs in aqueous media. Another assay (Swift and Guillard, 1977) to measure B_{12} plus its major biologically active analogs was performed with the diatom *Bellerochea polymorpha* clone 675-d which responds to B_{12} and also to the B_{12} analogs pseudovitamin B_{12} (adenyl cobamide cyanide) and Factor B (dicyanocobinamide). Because B_{12} and most of the analogs are cobamides, the term cobamide assay is used. The assay result is sometimes described as cobamide activity, meaning that it is a measure of the biological activity of several different chemical forms present in the sample. The exact amount of each in the sample is not known, but the reference for the assay is the exact concentration of a standard which produces the same biological response. The assay gives 120% response to Factor B and 40% response to pseudovitamin B_{12} relative to the response to B_{12} at the same concentration. If equal amounts of each of these two analogs and B_{12} are present, then the assay would measure 87% of the total concentration and the ratio of total cobamides : B_{12} concentration calculated from cobamide assay and B_{12} assay would be 2.6 rather than 3.0 (the calculated value). Further description of B_{12} terminology (Smith, 1965) and B_{12} specificity of organisms and assays can be found elsewhere (Guillard, 1968; Provasoli and Carlucci, 1974; Swift, 1980).

Summer and winter samples used for internal standards had greater initial B_{12} content and were not really suitable for construction of a standard curve by graded additions of B_{12}, because much of the curve was beyond the initial linear range of a B_{12} dose-response curve. (Slope values were 56.2 and 59.7 $\times$ 10^4 cells pg^{-1}, respectively, for summer and winter internal standard curves.) As a consequence, all data were analyzed with the external standard curve (prepared in water made vitamin-free by charcoal treatment). Slope of this curve is 69.7 $\times$ 10^4 cells pg^{-1}, which was not significantly different from that of the internal standard curve for spring.

In assay of B_{12} and cobamides, all samples and standards were sterilized by autoclaving except as described below for bound B_{12} determination.

The filtrate of the autoclaved whole water samples was assayed for vitamin B_{12}. The vitamin B_{12} concentration in filtered sea water from the same sample was subtracted to give an estimate of particulate vitamin B_{12}.

Bound vitamin B_{12}. Analysis for vitamin B_{12} was modified slightly so that samples could be assayed with and without autoclaving (to destroy binding factor). The thawed sample was divided ino two 50 ml subsamples. One was filtered through 0.22 μ membrane filter (GS, Millipore Corp.) using aseptic procedure. The other was autoclaved in a beaker-capped 125 ml glass flask. To each of these samples sterile nutrients were added (N, P, Si and trace metal mix). Added silicate was reduced to one-third that normally used, to avoid differential precipitation problems. Sample from each flask was aseptically dispensed in 10 ml portions into each of three sterile 13 $\times$ 125 mm culture tubes with plastic snap closures.

Autoclaving increases the vitamin B_{12} available in a sample containing binding factor. But autoclaving itself decreases the vitamin B_{12} activity at low concentrations as are found in sea water (Smith, 1965; Swift and Guillard, 1977). Thus standards are necessary to determine the effect of autoclaving on the vitamin activity, described by a factor Z, usually less than 1. Standards were made using vitamin-free Gulf of Maine sea water (Swift and Guillard, 1977). (The treatment also makes it free of organic materials such as proteins.) For one set of standards the water was autoclaved in flasks, following which sterile nutrients were added and sterile vitamin B_{12} was added. Portions were dispensed as were the samples. For the other set, vitamin-free water in flasks received 0, 0.1, 0.2, 0.3 or 0.4 ng l^{-1} vitamin B_{12} before autoclaving. Following the autoclaving, sterile nutrients were added and the water was dispensed.

All tubes were inoculated with *T. pseudonana* clone 3H prepared by growth in vitamin-free medium after previous growth with 5 ng l^{-1} vitamin B_{12} present. Initial cell density was 900 cells ml^{-1}. Cultures were incubated at 15C in continuous light and stirred daily. Cell yield was determined after eight days.

Data analysis was as follows. The effect of autoclaving is factor Z, ratio of slope for standard curve (with vitamin autoclaved) to that for standard curve derived without autoclaving the vitamin. The mean cell count, A, for the three replicates of each autoclaved sample was computed, as was the mean, B, for the sterile-filtered sample replicates. The fraction of B_{12} bound is $1 - \frac{BZ}{A}$.

Thiamin assay. Procedure was modified from that of Carlucci and Silbernagel (1966b) which utilizes *Pavlova lutheri* (=*Monochrysis lutheri*) a marine haptophyte requiring thiamin and B_{12}. They developed the method to utilize C^{14} uptake after two or three days incubation or cell counts after five days incubation. Here cell counts were used as the assay response, with smaller inocula and longer incubation times employed.

Stock cultures were maintained in tubes of vitamin-free f/2 medium (Swift and Guillard, 1977), with addition of 50 ng l^{-1} thiamin and 625 ng l^{-1} vitamin B_{12}. They were transferred at fortnightly intervals and inocula for assay were prepared by dilution with vitamin-free sea water or medium to yield initial density of several hundred to 1000 cells ml^{-1}, rather than 12,000 to 25,000 ml^{-1} as used by Carlucci and Silbernagel (1966b).

Assay cultures were grown in 20 × 150 mm screw cap culture tubes prepared as in the assay for cobamides (Swift and Guillard, 1977). Vitamin B_{12} (1 μg l^{-1} was added to all samples and standards. Each sample was prepared with three replicates, to one of which 10 ng l^{-1} was added as an internal standard to test whether the sample was toxic if no growth resulted in the other two tubes. In addition, three tubes were prepared in the same way with the sample diluted to 50% strength with vitamin-free sea water.

A series of external standards was prepared using vitamin-free Gulf of Maine water with thiamin additions of 0, 5, 10, 15, 20, 25, 30, 35 and 50 ng l^{-1}. A series of internal standards was prepared using Gulf of Maine surface water from the winter season with addition of 0, 5, 10, 15, 20, 25, 30, and 35 ng l^{-1}.

Samples were sterilized by autoclaving. Although thiamin is known to be degraded at high temperature (Gold, Roels and Bank, 1966), under the conditions used here 89% of thiamin activity remains for *M. lutheri* after autoclaving (Swift, 1973, 1980).

Cultures were incubated seventeen days with 0.042 ly min^{-1} illumination at 18C (13:11 LD cycle). Counting procedures were the same as for the vitamin B_{12} assay. The two series of standards gave slightly different values for the slope from linear regression analysis, 1.34×10^4 cells pg^{-1} for the external standard curve and 1.59×10^4 cells pg^{-1} for the internal standard curve. Thus the slope for internal standards was used in analyzing assays of undiluted samples, and the mean of the two slopes was used for samples diluted 50%.

Biotin assay. Biotin assay was modified from that of Carlucci and Silbernagel (1967) in the same way as was the thiamin assay. The assay organism *Amphidinium carterae* requires B_{12}, thiamin, and biotin. Stock cultures were maintained in moderate biotin concentration, 100 ng l^{-1}, and excess B_{12} (625 ng l^{-1}) and thiamin (2.5 μg l^{-1}). Stock cultures were started at two week intervals, using a four week old culture as inoculum. Assay cultures were inoculated from a stock culture to give initial density of 200-450 cells ml^{-1}.

Samples were prepared for assay with three tubes each at full strength and at 50% strength. To one tube of each, 4 ng l^{-1} biotin was added as internal standard to test possible toxicity. All samples and standards received addition of excess B_{12} and thiamin.

External standards were prepared in charcoal-treated Gulf of Maine sea water with biotin addition of 0, 0.5, 1, 2, 4, 6, 8, 10, 14, and 18 ng l^{-1}. Internal standards were prepared using surface sea water from the winter season. Autoclaving was used for sterilization, as activity remaining after autoclaving is high (84%), (Swift, 1973), and both samples and standards were treated the same. Tubes were incubated under the same conditions as thiamin assay for approximately 20 days.

3. Results

Vitamin B_{12}. Table 2 gives the results of vitamin B_{12} assay. Figure 3 shows vitamin B_{12} in different seasons. Winter concentrations are highest, 0.9 to 1.6 ng l^{-1}. During the spring bloom, the concentrations have decreased markedly. There is a decrease in the total vitamin B_{12} in the upper 100m during the spring bloom and the lowest concentration is 0.1 ng l^{-1}. Station 2, from the same region in August, resembles Station 822A, the earliest of the spring stations, but there is more vitamin B_{12} in

Table 2. Vitamin B_{12} assay.

Sample	B_{12}, ng l^{-1} ± s.d.	Sample	B_{12}, ng l^{-1} ± s.d.
Winter		Spring	
Station 1		855B	
1m	0.89 ± .08	1m	0.13 ± .03
20m	1.57 ± .10	10m	0.41 ± .04
40m	1.35 ± .15	20m	0.47 ± .02
60m	1.3 ± .2	30m	0.31 ± .03
		40m	0.24 ± .03
		100m	0.6 ± .1
Spring		Summer	
822A		Station 2	
1m	0.29 ± .02	5m	0.30 ± .03
20m	0.60 ± .07	25m	0.54 ± .06
50m	0.76 ± .08	50m	0.9 ± .1
100m	0.96 ± .1	100m	1.26 ± .07
		150m	1.7 ± .2
Spring		Summer	
840B		Station 3	
1m	0.45 ± .08	5m	0.80 ± .07
20m	0.41 ± .04	25m	1.3 ± .1
40m	0.46 ± .06	50m	1.36 ± .15
100m	0.55 ± .09	100m	1.9 ± .2
		135m	1.4 ± .1

deeper water samples in summer. The deepest samples contain as much as the winter samples. Station 3, which is another summer station, contains higher B_{12} concentrations down to 100m.

Particulate vitamin B_{12}. Table 3 shows dissolved + particulate vitamin B_{12} concentrations measured at surface and 20m at the stations during the spring. The particulate B_{12}, determined by subtraction, is greater than dissolved vitamin B_{12}. Vitamin B_{12} per unit particulate carbon was also calculated. Values were 5 to 10 ng B_{12} per mg C with the exception of the high 20m sample, Station 822A, considered aberrant, probably from non-representative distribution of particulate matter.

Cobamide concentration. Results of cobamide assay are given in Table 4. Figure 4 shows the seasonal variation, which has the same general trends as vitamin B_{12}. Table 5 gives the ratio of the cobamide : B_{12} concentrations. All values are approximately 1 or greater with the exception of one sample where coefficient of variation for one of the assays was ± 100%. The mean values for cobamide : B_{12} ratio at winter and summer stations are 2.4 and 2.3, respectively, and 1.4 for spring samples.

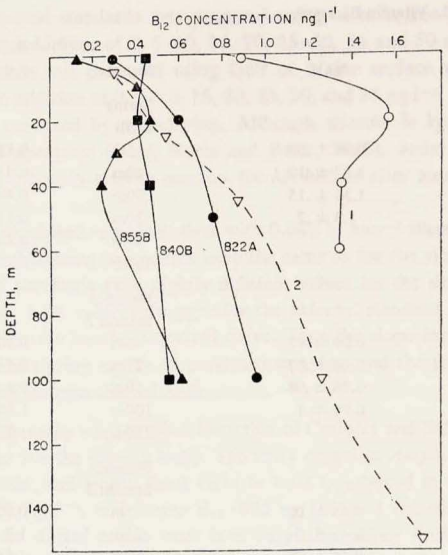

Figure 3. Vitamin B_{12} in the Gulf of Maine. Open circles: Station 1 (Winter). Closed circles: Station 822A (April 4). Squares: Station 840B (April 10). Closed triangles: Station 855B (April 15). Broken line: Station 2 (Summer).

Table 3. Vitamin B_{12} content of particulate matter during spring bloom.

Station	Depth	Dissolved + Particulate B_{12} ng l^{-1}	Particulate B_{12} ng l^{-1}	Partic. Carbon* mg l^{-1}	Partic. B_{12}/Partic. C ng mg^{-1}
822A	1m	1.16 ± .06	0.87	139.0	6.3
	20m	3.9 ± .1	3.3	89.6	37
840B	1m	1.1 ± .2	0.65	116.4	5.6
	20m	1.27 ± .08	0.86	90.4	9.5
855B	1m	1.4 ± .3	1.3	194.5	6.7
	20m	1.8 ± .4	1.3	146.0	8.9

* Dr. B. H. Ketchum and N. M. Corwin (Woods Hole Oceanographic Institution), personal communication.

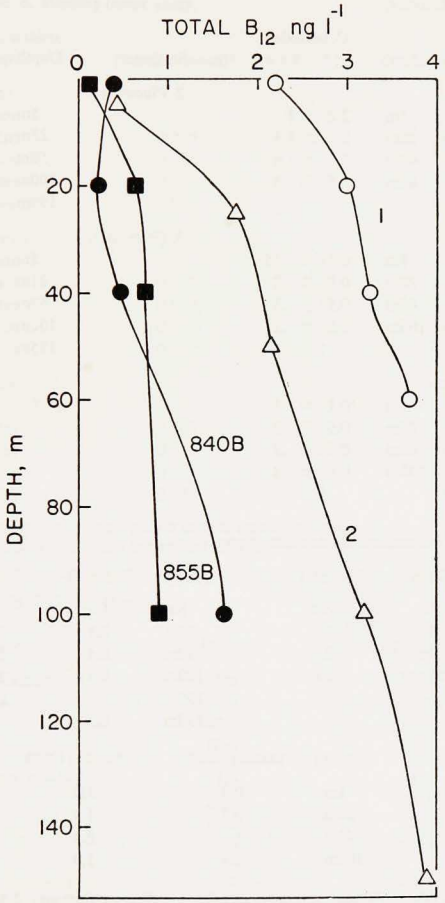

Figure 4. Cobamide concentration (B_{12} + analogs) in the Gulf of Maine. Open circles: Station 1 (Winter). Closed circles: Station 840B (April 10). Squares: Station 855B (April 15). Triangles: Station 2 (Summer).

Table 4. Cobamide assay.

Station	Depth	Cobamides ng l^{-1} ± s.d.	Station	Depth	Cobamides ng l^{-1} ± s.d.
1 (Winter):			2 (Summer):		
	1m	2.2 ± 1		5m	0.43 ± .15
	20m	3 ± 1.3		25m	1.8 ± .5
	40m	3.2 ± .6		50m	2.2 ± .8
	60m	3.7 ± .3		100m	3.2 ± .5
				150m	3.9 ± .8
840B (Spring):			3 (Summer):		
	1m	0.41 ± .15		5m	1.5 ± .4
	20m	0.2 ± .2		25m	2.5 ± .6
	40m	0.5 ± .5		50m	3.08 ± .05
	100m	1.6 ± .2		100m	4.2 ± 1
				135m	3.6 ± .6
855B (Spring):					
	1m	0.1 ± .1			
	20m	0.6 ± .2			
	40m	0.7 ± .7			
	100m	0.8 ± .2			

Table 5. Ratio of cobamides: B_{12} concentration.

Winter	Station 1	Summer	Station 2	Station 3
1m	2.4	5m	1.4	1.9
20m	1.9	25m	3.3	1.9
40m	2.4	50m	2.4	2.2
60m	2.8	100m	2.5	2.2
		135m		2.5
		150m	2.3	

Spring	Station 840B	Station 855B
1m	0.9	1.0
20m	0.5	1.3
40m	1.0	2.9
100m	2.9	1.3

Mean Values Spring 1.4 Winter/Summer 2.3

Binding factor assay. Table 6 shows the results for binding factor assay. The autoclaving factor, described previously, was 0.65 in this set of assays. The mean coefficient of variation for the cell crop measurements was 10%. In a number of samples the fraction of B_{12} bound is significantly different from 0.

Table 6. Binding factor assay.

Season, station, depth	Fraction bound*	B_{12}* ng l^{-1}	Bound B_{12} ng l^{-1}
Winter			
Station 1			
1m	0.38*	0.89	0.34
20m	0.51*	1.57	0.80
40m	0.2	1.35	0.27
60m	0.5 *	1.3	0.65
Summer			
Station 3			
5m	0.5 *	0.80	0.4
25m	0.60*	1.3	0.78
50m	0.60*	1.36	0.82
100m	0.65*	1.9	1.23
Spring			
Station 855B			
1m	0.2 *	0.13	0.03
20m	0	0.47	0
40m	0	0.24	0
100m	0.36*	0.6	0.22

* = Bound B_{12} significantly different from 0 at 95% confidence level.
* Free plus bound B_{12} as determined in standard bioassay.

Table 7. Thiamin assay.

Station	Depth	Thiamin concentration ng l^{-1} ± s.d.
1 (Winter)	1m	14 ± 5
	20m	23 ± 5
	40m	16 ± 4
	60m	21 ± 6
855B (Spring)	1m	10 ± 4
	20m	18 ± 8
	40m	10 ± 2
	100m	10 ± 6
3 (Summer)	5m	34 ± 7
	25m	40 ± 18
	50m	46 ± 17
	100m	45 ± 14

Thiamin. Thiamin concentrations vary from 10 to 46 ng l^{-1} (Table 7). Variations in cell counts result in an average coefficient of variation of 35%. The variations are random and not associated with differences in results between the 50% and

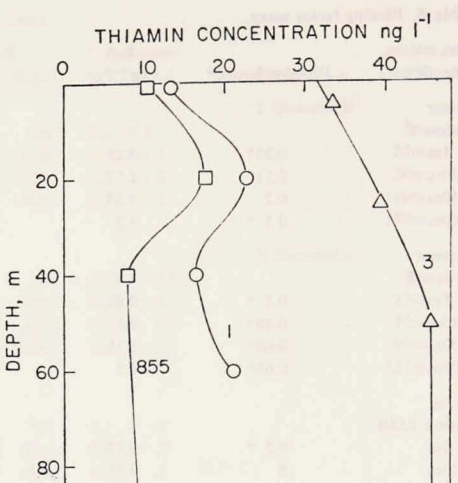

Figure 5. Thiamin in the Gulf of Maine. Circles: Station 1 (Winter). Squares: Station 855B (Spring). Triangles: Station 3 (Summer).

100% sample strengths. There was inhibition apparent in only one sample, 100m at Station 3. Here the concentration detected in the 100% tubes was less than half that measured in the 50% series, and the latter was used for the determination.

Figure 5 shows thiamin concentrations in different seasons. Concentrations decrease slightly from winter to spring. In summer concentrations increase greatly.

Biotin assay. Inhibition was present in all samples assayed without dilution. This was exhibited by the same amount of growth or less growth than in the diluted samples; in some cases there was almost no growth. In replicates of undiluted samples or those diluted 50% there was close agreement in cell density regardless of whether or not inhibition was present.

Biotin concentration was calculated using the three tubes at 50% dilution. The mean of the cell density in the two samples without added biotin was subtracted from the cell density in the tube with 4 ng l^{-1} and used to estimate biotin in the sample tubes. The calculated carryover of biotin in the inoculum, 1 ng l^{-1}, was then subtracted. There was inhibition in these samples also, as exhibited by more than two-fold variation in the additional growth produced by the addition of 4 ng l^{-1}

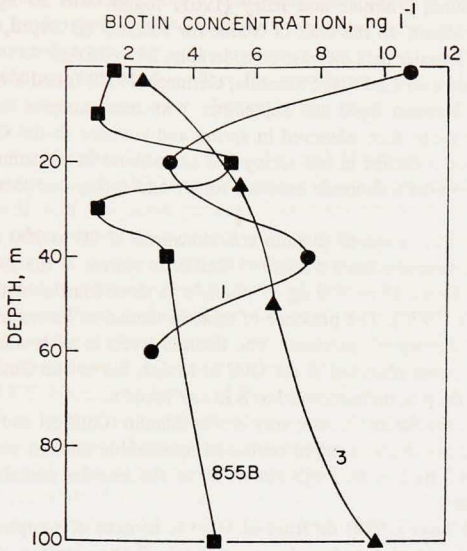

Figure 6. Biotin in the Gulf of Maine. Circles: Station 1 (Winter). Squares: Station 855B (Spring). Triangles: Station 3 (Summer).

biotin. This was a directional trend in that those samples containing the greatest amount of biotin gave the least additional growth in the internal standard. Because the series of standard curves could not be used, it is difficult to state precision. Counting error is small relative to variation in yield in the different internal standards; thus a rough estimate of precision is ± 50%, based on use of the internal standards and not on reproducibility in individual samples.

Computed biotin concentrations are shown in Figure 6 and vary from 1 to 11 ng l^{-1}. Samples from 10m and 30m at Station 855 were assayed on a different occasion from the bulk of the samples at greater dilution, and inhibition was not apparent. The calculated coefficient of variation for these is 30%.

4. Discussion

Thiamin. Results for thiamin and biotin assay will be discussed briefly first, and then emphasis will be on B_{12} in the remainder.

The thiamin concentrations can be compared with those in other regions. In

Long Island Sound, Vishniac and Riley (1961) found 0 to 20 ng l^{-1} between November and March. In the Gulf of Maine for January and April, values are 10 to 23 ng l^{-1}; in summer they increase considerably, 34 to 46 ng l^{-1}.

In coastal waters off La Jolla, California, Carlucci (1970) found a range of values of 2-25 ng l^{-1} between April and September, with most samples containing 5-10 ng l^{-1}, a lower range than observed in spring and summer in the Gulf of Maine. Carlucci observed a decline in late spring and an increase in the summer. The Gulf of Maine water shows a decrease between winter and spring and then a substantial increase.

Natarajan (1968) measured thiamin concentrations of 20 to 200 ng l^{-1} in 6 of 16 surface water samples from southeast Alaska. In waters of the subarctic Pacific near Alaska he found 13 to 400 ng l^{-1} thiamin in three-fourths of the 42 samples from 20 stations (1970). The presence of thiamin tended to be associated with low concentrations of inorganic nutrients. The thiamin levels in such waters tend to be higher than the range observed in the Gulf of Maine, but in the Gulf of Maine the thiamin did not drop to undetectable levels in any samples.

Samples from the Antarctic were very low in thiamin (Carlucci and Cuhel, 1975). Only 7% of water samples assayed contained measurable thiamin and the range of values was 0 to 7 ng l^{-1}, although almost all of the samples contained thiamin in particulate matter.

Ohwada and Taga (1972) determined vitamin content of samples from several areas near the coast of Japan and from several offshore cruises. At Aburatsubo Inlet of Sagami Bay, mean surface values for spring, summer, and autumn were 40, 92, and 23 ng l^{-1}, respectively. Concentrations between December and February averaged 6 ng l^{-1}. Water from the bottom layer in all seasons contained slightly less thiamin. In Sagami Bay itself, during spring, thiamin averaged 5 ng l^{-1} in the 0 to 20 m layer. In summer the maximum values occurred, with a mean of 39 ng l^{-1}, followed by a drop to mean value 7 ng l^{-1} in fall, and no detectable thiamin present between December and February. From 20 to 200 m no thiamin was detected at any season. The general seasonal variation of thiamin in these coastal regions followed the same trend as chlorophyll *a* concentrations. The concentrations in the Gulf of Maine are greater than those of Sagami Bay, but less than those observed in the Aburatsubo Inlet. While the summer concentration is similar to that in Sagami Bay, the existence of detectable thiamin in winter and in samples below 20m makes the Gulf of Maine differ from this coastal area near Japan.

In samples offshore, Ohwada and Taga (1972) found a mean value of 3 ng l^{-1} in samples taken between 0m and 20m in the North Pacific. No thiamin was detected in 76 samples from greater depths. In a total of 59 samples from 12 stations in the East China Sea, no thiamin was detectable.

Biotin assay. Biotin values should be considered approximate because of the inhibi-

tion observed. When inhibition occurs, one assumption made in performing bioassays no longer holds: that all nutrients are in excess except the one under analysis and growth response depends only on the amount of that nutrient. With the presence of inhibition, unknown factors also affect the growth response and an additional assumption is necessary: inhibition is identical in the sample and the sample with internal standard added.

The assay of two samples (Station 855B, 10m and 30m) on a different occasion with greater dilution and no inhibition, gave results in the same range of magnitude as those measured with inhibition present. This gives some confidence that the estimated biotin values represent the proper order of magnitude.

The inhibition may have been in part an artefact of the sample preparation rather than sample content because it was not observed consistently. Precipitates might have formed in the medium, and dinoflagellates seem to be sensitive to such phenomena in that procedures which decrease particulate matter lead to better success in growing dinoflagellates (Brand, Guillard and Murphy, 1981; Siegelman and Levandowsky, 1979; Harrison, Waters and Taylor, 1980).

There is a drop in biotin concentration from the winter to the spring bloom. Values are higher in summer, showing a gradual increase with depth.

In comparison with other regions, values are slightly higher than in Sagami Bay of coastal Japan (Ohwada, 1972) in the upper layer and substantially greater in the deeper samples; in the Gulf of Maine samples from 40m and below have 3-10 ng l^{-1}, whereas samples below 20 m in Sagami Bay had less than 1 ng l^{-1}. A similar generalization holds true for comparison with oceanic stations in the East China Sea and the North Pacific Ocean (Ohwada and Taga, 1972) and in the north Pacific (Carlucci and Silbernagel, 1967). Surface values in the Gulf of Maine in spring and summer are similar to those in coastal water off La Jolla for the period April to September (Carlucci, 1970).

Natarajan (1968) analyzed samples from southeast Alaska waters, and Litchfield and Hood (1965) assayed samples from the Gulf of Mexico. Both studies found biotin lacking in the majority of samples and many of these samples were inhibitory or toxic to the bioassay bacterium. In the remainder of the samples, values of 1-4 ng l^{-1} were found.

Vitamin B_{12}—geographical comparison. B_{12} concentrations range from 0.1 to 1.9 ng l^{-1}. This is lower than in the nearest coastal regions studied previously, Long Island Sound with 4-16 ng l^{-1} (Vishniac and Riley, 1961) and Napeague Bay off eastern Long Island with values of < 0.05 ng l^{-1} to 11 ng l^{-1} (Bruno and Staker, 1978). The range was 0 to 3 ng l^{-1} at the most offshore station adjacent to Block Island Sound, but this was still quite close to shore with only 16 m depth. Other more distant coastal regions also have higher concentrations, 1-16 ng l^{-1} in the Strait of Georgia, British Columbia (Cattell, 1969, 1973), and 0.6 to 4.8 ng l^{-1}

for seasonal mean values in Sagami Bay, Japan (Ohwada and Taga, 1972). B_{12} values in the Gulf of Maine are similar to those of stations in the subarctic Pacific which has 0 to 2 ng l^{-1} (Natarajan, 1970) and greater than in the Sargasso Sea where Menzel and Spaeth (1962) measured 0.01 to 0.1 ng l^{-1} in most of the samples from 200m or less. Seasonal distribution of B_{12} in the Gulf of Maine resembles that in the Sargasso Sea in the presence of maximum values in winter, followed by a decline during the spring bloom. All of the studies mentioned above were assayed with organisms showing strict specificity for B_{12}.

B_{12} assays of Carlucci and co-workers use *T. pseudonana* clone 13-1 which has a 60% response to pseudo B_{12} and Factor *B* (Carlucci and Silbernagel, 1966a), thus measuring in B_{12} and analogs more than the 3H assays used here but less than the 675-d cobamide assay used for the Gulf of Maine, which yielded a range of 0.1 to 4 ng l^{-1}. Slightly higher B_{12} was found off La Jolla, California, 0.4-6.5 ng l^{-1} (Carlucci, 1970) whereas the upper 200m of oceanic stations were similar to the Gulf of Maine, 0 to 3 ng l^{-1} in the California current and 0 to 2 ng l^{-1} at stations in the eastern Central Pacific (Carlucci and Silbernagel, 1966c). Samples from the Southern Indian Ocean (Carlucci and Cuhel, 1975) were notably low in B_{12}, despite high concentrations of inorganic nutrients. From stations south of the Antarctic Convergence, almost all samples from the upper 250m contained 1 ng l^{-1} or less. One-third of samples in the upper 75m had no detectable B_{12}, and 0.2 ng l^{-1} was the average value. Even samples lacking dissolved B_{12} had particulate B_{12}, with particulate + dissolved B_{12} showing a range of 0.15 to 0.56 ng l^{-1} in eight samples tabulated. The ratio B_{12} : C in particulate matter was 2.5 ng B_{12}/mg C for those samples, whereas in the Gulf of Maine the particulate + dissolved B_{12} is 1-4 ng l^{-1} in spring, and B_{12} content was 7.4 ng B_{12}/mg C. Thus the Gulf of Maine contains more vitamin B_{12} than Antarctic waters and three times as much B_{12} per unit carbon in particulate matter, suggesting plankton in both regions is taking up external vitamin B_{12}, with Gulf of Maine plankton less likely to be limited by B_{12}. The Antarctic samples are dominated by diatoms but it is not known whether these species require B_{12} from an external source.

Thus many coastal regions contain greater vitamin B_{12} concentrations than the Gulf of Maine, which more resembles oceanic regions except for those notably poor in all nutrients (such as the Sargasso Sea) and the Antarctic, which is unusual in being very low in B_{12} and thiamin in the presence of high concentrations of other nutrients.

The vitamin levels measured are a sampling of different seasons; however they do not represent an actual annual cycle because they were not taken consecutively and frequently. The first samples and the algal isolations were made during the spring phytoplankton bloom. Because of success in sampling the bloom, additional water samples were taken the following August and January. However loss of

August samples in storage made it necessary to replace these two years later. The January samples were taken further inshore than the others, but literature data show uniform temperature and nutrient conditions throughout the Gulf of Maine in winter (Bigelow, 1926; Bigelow, Lillick and Sears, 1940; Gran and Braarud, 1935).

Particulate B_{12}. During the spring bloom, particulate B_{12} is greater than dissolved B_{12}. The initial reason for assaying some samples for particulate B_{12} was to detect presence of the vitamin if B_{12} were undetectable in the dissolved portion. The values for particulate + dissolved B_{12} in spring are in the same range as dissolved B_{12} alone during winter. This raises the possibility of conservation of B_{12} during the period when temperatures are low, that almost all of the B_{12} used during early stages of the bloom is present before it starts.

The vitamin content of most spring bloom samples was 5-10 ng B_{12}/mg C. Carlucci and Bowes (1972) measured 0.3 to 1 ng B_{12}/mg C in autotrophic algae (*Coccolithus huxleyi* and *Phaeodactylum tricornutum*) grown without added B_{12}. *Skeletonema costatum*, a B_{12} auxotroph, contained 5-6 ng B_{12}/mg C in a first depletion culture (300 ng l^{-1} total dissolved + particulate B_{12}) after growth at 100 ng l^{-1} B_{12}. In a second depletion culture cells contained 0.5-0.7 ng B_{12}/mg C (total B_{12} 9.5 ng l^{-1}). Thus values for B_{12} content of Gulf of Maine phytoplankton are in excess of those of autotrophic algae producing their own B_{12} and closely resemble values for *S. costatum* growing with excess available.

If detritus is present in the sample for particulate carbon, then the actual ratio for phytoplankton cells is greater. In addition, samples were autoclaved initially to extract B_{12}. They were autoclaved again for assay, as were the standards. Vitamin B_{12} is probably underestimated slightly because loss of B_{12} from autoclaving twice is slightly greater than for a single time.

Cobamide concentration. Cobamide concentrations show a general seasonal pattern similar to those of B_{12}. The ratio cobamide concentration : B_{12} concentration (Table 5) shows seasonal variation. The one winter and two summer stations have a mean value of 2.3 whereas the two spring stations have 1.4 mean value. Thus in winter and summer more biological activity is present in non-B_{12} cobamides than in vitamin B_{12}, whereas in spring most of the activity is in vitamin B_{12}. In some oceanic samples Cowey (1956) found very little analog activity, but in samples from Aberdeen Bay there was perhaps as much activity attributable to the pseudo B_{12} class of analogs as to cyanocobalamin. In sediment samples and bacterial cultures, the major part of cobamide activity is in compounds other than true vitamin B_{12} (Starr, Jones and Martinez, 1957; Burkholder and Burkholder, 1958). Richness in analogs in winter and summer might be associated with B_{12} originating in the organic-rich sediments, which act as a vitamin source for shallower water through mixing. B_{12} production is also associated with bacteria from the upper layer of water (Berland,

Bonin, Durbec and Maestrini, 1976; Lebedeva, Markianovich and Gutveyb, 1971; Vacelet, 1975; Haines, 1972, 1974, Haines and Guillard, 1974). However, there is little information about their differential production of B_{12} and analogs.

If the main sources of non-cobalamin cobamides are bacteria, these include (1) benthic bacteria, whose release of vitamins reaches the upper depths through vertical mixing, and (2) *in situ* bacterial production. In winter the water column is vertically well-mixed so cobamide concentration is high throughout. In summer, there is high cobamide concentration in deeper water, and slightly less above 60m because stratification lessens vertical mixing but higher temperatures allow *in situ* release by bacteria. In the spring the water column is beginning to stabilize, so there is less input of (B_{12} + analogs) from mixing. The analogs that are used biologically are not replenished. Bacterial activity should be low because of low temperature and because algal cells are not yet in the senescent state in which most organic excretion takes place. Dissolved B_{12} concentration is the net result of those processes which supply B_{12} and those which remove it. Because B_{12} decreases slowly during the bloom, and much B_{12} goes into particulate material, it seems logical to assume that B_{12} is not being added at a significant rate. Algae can be ruled out as a significant source of B_{12} because they are taking it up at this time. With bacterial activity thought to be low, a remaining possible source is zooplankton activity. Zooplankton at that time (T. Lawson, personal communication) has need of only 1-3% of the algal organic carbon per day, so its effect on phytoplankton biomass is not yet significant. However, the action of zooplankton feeding in an axenic rotifer-alga system releases about 30% of the B_{12} in ingested algae as soluble B_{12} (Droop and Scott, 1978). Such an effect could add 0.006 ng B_{12} l^{-1} each day, which could be taken up again for algal growth. Results of such zooplankton activity would be to favor recycling of B_{12}, rather than analogs, because most algae use B_{12} preferentially to analogs (Guillard, 1968).

Bruno and Staker (1981) assayed water samples from Peconic Bay, Long Island, a shallow well-mixed estuary, using *T. pseudonana* clones 3H (B_{12} response only) and 13-1 (50% response to pseudo B_{12} and Factor *B* as well). The ratio of results of the two assays varied greatly, and B_{12} and cobamide peaks occurred at different times. This suggested benthic bacteria were a major source of B_{12} and cobamides, with these substances released in different proportions by various strains at different times.

Bound B_{12}. Besides assaying for B_{12} and cobamides, it was considered desirable to test for presence of bound B_{12} in water samples. Heat-labile proteins that bind B_{12} have been found in medium of mature cultures of algae (Kristensen, 1955, 1956; Ford, 1958; Droop, 1968; Pintner and Altmyer, 1979) and in chemostat cultures of marine algae (Droop, 1968, 1970). Binding factor production in culture is mainly dependent on cell density (Pintner and Altmyer, 1979; Droop, 1968), and binder

concentration in cultures can greatly exceed the B_{12} concentrations of natural waters. Application of such data to the field is difficult because of much lower magnitude of cell density, growth rate, other nutrient concentrations etc. Most workers have found small amounts of vitamin B_{12} to be present even when water samples were prepared under conditions that do not destroy binding proteins (Carlucci and Silbernagel, 1966c; Bruno and Staker, 1978). Pommel (1975) compared results for B_{12} assay in autoclaved and non-autoclaved samples from Lake Geneva using *Euglena gracilis assay*. There was indication of slightly higher activity in some samples after autoclaving, but it was not statistically significant. Pommel concluded that B_{12} was present in unbound form.

Results in Table 6 indicate most samples in winter and summer have 40-60% bound B_{12} (0.3 to 0.8 ng l^{-1}). This is in contrast to the higher values observed in cultures, 20 to 60 ng l^{-1} (Pintner and Altmyer, 1979). Spring samples lack significant bound B_{12} or, if it is statistically significant, the absolute amount is very low. It is likely that some of the same factors are involved for binding factor as for cobamide concentration in the occurrence of higher values in winter and summer samples. Spring samples have little or none, indicating the *Thalassiosira* bloom did not excrete significant amounts of binding factor while taking up B_{12}. Experiments with cultures support this (Swift and Guillard, 1978). In addition, binding factor is less stable than B_{12} and exposure even to low temperatures (3C-5C) causes denaturation after more than a month or two (Pintner and Altmyer, 1979). Sunlight and surface agitation may also contribute to denaturation, and proteolytic bacteria can degrade the protein rapidly. Thus environmental conditions promote loss of binder activity during periods when rate of supply (through sources such as vertical mixing) decreases.

Ecological role of vitamins. At the time when the spring water samples were taken, the bloom was underway, and vitamin B_{12} concentrations were substantially less than winter levels, presumably because of consumption by the diatom population. At the three stations made at one week intervals during the spring bloom, the totals of dissolved vitamin B_{12}, integrated over the upper 100 m, steadily decreased (Fig. 3). Significant vitamin B_{12} is present in the particulate matter (Table 3). So it appears that the spring bloom in the Gulf of Maine is a vitamin consuming bloom. In the laboratory it takes considerable time for senescent cells to begin growth without B_{12}. Clones of *Thalassiosira* spp. from the Gulf of Maine utilize external vitamin B_{12} when it is present and are able to grow to a bloom population density (defined for the Gulf of Maine as 10^6 cells l^{-1}) 4 to 54 days sooner than cells inoculated into vitamin-free medium (Swift and Guillard, 1978). Cells lacking a B_{12} supply can synthesize it at the cost of a long lag phase and lower growth rate.

It seems likely that the B_{12} pathway is not operating in cells that initiate the bloom, whether these are senescent vegetative cells or resting spores. It is reason-

able to conclude that the high concentration of B_{12} present before the bloom promotes development of the bloom. By utilizing an external supply of B_{12}, *T. nordenskioeldii* and other diatoms can avoid a long lag period to initiate vitamin B_{12} synthesis. When the physical and chemical environment is suitable for cell multiplication, the population can develop.

The extent to which B_{12} eventually becomes depleted in the upper layer before *T. nordenskioeldii* passes from dominance and *Chaetoceros* species take over is not known. Observations during the bloom lasted only two weeks, at the end of which B_{12} was 0.1 ng l^{-1}, a level which would prevent maximum growth rates in B_{12} auxotrophs such as *T. pseudonana, P. lutheri,* and *S. costatum* which have K_s values of 0.2 to 0.4 ng l^{-1} (Swift and Taylor, 1974; Carlucci and Silbernagel, 1969, Droop 1970). A clone of *Chaetoceros concavicornis* showed B_{12} stimulation typical of the faculative vitamin autotrophy of *T. nordenskioeldii,* as did *T. aestivalis, T. rotula,* and *Porosira glacialis* (Swift and Guillard, 1978).

At Station 2 (summer), levels of B_{12} remained as low at 5m and 25m as the spring values, but steadily increased with depth. Station 3 (summer) shows the same pattern except all values are about 0.4 ng l^{-1} greater, and in general all stations show increased B_{12} at depth. As with inorganic nutrients, it appears higher B_{12} concentrations occur in deep water or sediments, and vertical mixing is important in replenishing the upper layer. If Station 2 (summer) has the same fraction of B_{12} bound as Station 3, then free B_{12} concentration at the surface is 0.15 ng l^{-1}, the same as was present at the end of the spring bloom. Dominant organisms in summer are dinoflagellates and diatoms. *Skeletonema costatum* is the dominant diatom, but it is more important in coastal waters than offshore. *S. costatum* isolated by Haines (1972) in summer from a sample taken near Station 2 requires vitamin B_{12}, as do clones from other regions. B_{12} in summer is not sufficient to produce maximum growth rates for B_{12} auxotrophs, but it is sufficient to allow growth and is greater than B_{12} present at the start of the spring flowering in the Sargasso Sea which produces up to 250,000 diatom cells l^{-1} and depletes the water of B_{12} (Menzel and Spaeth, 1962; Hulburt, Ryther and Guillard, 1960).

The present study did not include organisms other than diatoms. Many dinoflagellates (23 of 26 reported by Provasoli and Carlucci in 1974) do require vitamin B_{12} alone or in combination with other vitamins. In the Strait of Georgia, B. C. (Cattell, 1969), the summer dinoflagellate population was correlated with nitrogen not B_{12}, although there both B_{12} and dinoflagellate levels were 1-2 orders of magnitude greater than in the Gulf of Maine. In some years, the haptophyte *Coccolithus huxleyi* (a thiamin requirer) dominates in the Gulf of Maine in the summer, a period when thiamin is plentiful.

Some of the dinoflagellates that dominate in summer might have an absolute requirement for biotin. Four out of 18 species from other regions require biotin in culture (Loeblich, 1966). The biotin half-saturation constant for CO_2 uptake rate

by *Amphidinium carterae* is 4 ng l^{-1} (Carlucci and Silbernagel, 1969). Biotin concentrations of 2-5 ng l^{-1} found in the upper 25m in summer are insufficient to allow maximum photosynthetic rate in organisms having similar kinetics.

Vitamin B_{12} is the vitamin in least supply in the Gulf of Maine by comparison with other regions and examination of physiological parameters. The dominant phytoplankton species is *Thalassiosira nordenskioeldii*, a facultative autotroph for B_{12}. Maximum vitamin concentrations occur in winter, allowing the centric diatoms to initiate the spring bloom and avoid a lag by behaving as if they were B_{12} auxotrophs when other conditions are suitable for cell increase.

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