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Distribution of Thiamine, Biotin, and Niacin in the Sea[1]

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The distribution of thiamine, biotin, and niacin was investigated in surface waters collected from 20 stations in southeast Alaska, by use of bioassay methods. A marine yeast, *Cryptococcus albidus*, was used for thiamine, and mutants of the marine bacterium *Serratia marinorubra* were used for biotin and niacin. Thiamine was found in 6 (38%) of the samples in amounts ranging to 200 ng/liter; biotin was found in 8 (38%) of the samples in amounts ranging to 3.1 ng/liter. Niacin was found to be completely absent in all of the samples assayed. The significance of the presence of thiamine and biotin in the productivity of the area is discussed.

The importance of organic growth factors in the marine environment has been extensively discussed in recent review articles by Provasoli (8), Belser (3), Riley (10), and Hood (5). Among the vitamins, at the present time, only vitamin B_{12}, thiamine, biotin, and niacin have been found to be important in the biological productivity of the oceans, a deduction based mainly on laboratory studies of the nutritional requirements of pure cultures of marine microorganisms.

Although the presence of the above vitamins is indicated in the marine environment by the laboratory studies, we have very little data on the distribution of these vitamins except in the case of vitamin B_{12}. The distribution of thiamine has been investigated only by Vishniac and Riley (11) and Natarajan and Dugdale (7). Antia (1), Litchfield and Hood (6), Carlucci and Belser (Bacteriol. Proc., p. 3, 1963), Provasoli and Gold (9), and Belser (2) have developed bioassay methods for biotin with the analysis of few natural seawater samples. The only study of the distribution of niacin is that of Belser (3). The combined bioassay for all the four vitamins in one water mass is completely lacking. Vishniac and Riley (11) and Carlucci (Abstr. 11th Pacific Science Congr., Tokyo, vol. 2, p. 33, 1966) reported on the distribution of vitamin B_{12} and thiamine in Long Island Sound and the northeast Pacific Ocean, respectively. The significance of the presence or absence of physiologically active amounts of these vitamins in different parts of the ocean still remains to be assessed. The present study is the first attempt to bioassay for all three vitamins—namely, thiamine, biotin, and niacin—in one area (Southeast Alaska).

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MATERIALS AND METHODS

A thiamine bioassay method with the marine yeast *Cryptococcus albidus* (Saito) Skinner has been described by Natarajan and Dugdale (7).

Biotin assay with *Serratia marinorubra* A101 V mutant was the same as that employed by Litchfield and Hood (6), with the minor modifications described below. The set up for niacin bioassay with *S. marinorubra* S817 V mutant was also similar to that used in the biotin assay. The standard curves were made in charcoal-treated Rila marine mix (Rila Products, Teaneck, N.J.) adjusted to 31‰ salinity. The standards and the samples were made in duplicate culture tubes (18 × 150 mm, with Morton stainless steel closures; Bellco Glass, Inc., Vineland, N.J.), autoclaved for 15 min at 121 C, and cooled to room temperature overnight. In the case of natural seawater samples, one set of internal standards in duplicate were also included, with 10 pg of biotin per ml and 6 ng of niacin per ml.

After inoculation, the samples and the standards were mounted in wooden blocks and placed in a specially constructed rack in a New Brunswick Gyrotory shaker (model G-10) in a constant-temperature room maintained at 25 C. After the incubation periods, the results were read turbidimetrically by use of a Beckman DU spectrophotometer. *C. albidus* is sensitive in the range of 10 to 300 pg of thiamine per ml, and the optical density was measured at 450 mμ after an incubation period of 110 hr. The ranges of sensitivity for the biotin mutant A101 V and the niacin mutant S817 V of *S. marinorubra* are, respectively, 1 to 16 pg of biotin per ml and 1 to 30 ng of niacin per ml; the optical density of both was measured at 530 mμ after an incubation period of 72 hr.

Surface samples of seawater for the bioassay were collected during three cruises of R/V ACONA in

southeast Alaska: cruise 17 (11–20 November 1965), cruise 21 (19–24 March 1966), and cruise 22 (2–16 April 1966). The samples were collected with a 16-liter plastic bucket from the side of the ship with the least pollution and debris and rinsed once with the same seawater. A sample of about 1 liter was immediately filtered through a Hurlbut 984H glass filter paper, transferred to three plastic bottles, and stored frozen. The filtration unit was washed before filtering the final sample by sucking through and discarding part of the sample. The plastic bottles used were of 125-ml capacity and had been washed, rinsed in distilled water, and then rinsed in dilute HCl. The samples were transported to the laboratory in a frozen condition and were stored frozen until the time of bioassay. The samples were thawed in lukewarm water just before the bioassay.

RESULTS AND DISCUSSION

Twenty stations were sampled in southeast Alaska during the months of November 1965 and March and April 1966 (Table 1). Two stations were sampled at two different times of the year to determine the seasonal differences. Niacin and biotin were analyzed in 21 samples and thiamine in 16 samples. Six (38%) of the samples showed the presence of thiamine in amounts ranging to 200 ng/liter and eight (38%) showed the presence of detectable amounts of biotin ranging to 3.1 ng/liter (Table 1). Samples from 16 stations were analyzed for all the three vitamins together. Only one (6%) sample showed the presence of thiamine and biotin together, five (31%) showed thiamine alone, three (19%) showed biotin alone, and seven (44%) showed no vitamins. Niacin was completely absent in all samples.

In Lisianski Inlet (station 246), which was sampled twice at two different times of the year (November and April), all vitamins were absent during both sampling periods. Icy Strait (station 358), on the other hand, showed maximal concentrations of thiamine and biotin both in March and April. Icy Strait also has been found to be rich in thiamine during a previous investigation (7).

The absence of niacin in all samples analyzed may be attributed to the lack of the vitamin per se, or the expected range of concentration may fall below the dose-response curve of the *Serratia* mutant. This can be further checked only by bioassay of samples with a more suitable organism and with a lower range of sensitivity. The results show that detectable amounts of biotin are present in surface waters, although the range of concentrations found is many orders of magnitude less than that found for thiamine.

The *Serratia* mutants used were found to be very sensitive to some inhibitory factor(s) present

TABLE 1. *Distribution of thiamine, biotin, and niacin in surface waters of southeast Alaska*

Sampling date	Station no.	Latitude N	Longitude W	Thiamine[a] (ng/liter)	Biotin[a] (ng/liter)	Niacin[a] (μg/liter)
11–20 November 1965	219	58°16.7′	134°22.7′	20	u	u
	221	58°12.5′	134°06.7′	92.5	u	u
	225	56°03.0′	134°41.4′	62.5	u	u
	226	56°29.3′	134°30.0′	65	u	u
	228	57°08.1′	134°44.5′	200	u	u
	233	57°49.7′	135°24.8′	—	1.0	u
	246	57°58.6′	136°16.3′	u	u	u
	247	57°57.3′	136°22.6′	—	1.4	u
	257	58°44.4′	136°18.0′	—	1.6	—
	260	58°54.4′	136°58.2′	—	—	u
	262	58°55.0′	136°05.5′	—	u	u
19–24 March 1966	352	57°55.1′	133°32.0′	u	u	u
	358	58°19.2′	135°25.3′	200	0.9	u
	359	58°13.0′	134°58.5′	u	u	u
	360	58°30.4′	135°04.3′	u	1.0	u
2–16 April 1966	384	58°48.1′	134°37.7′	u	u	u
	391	57°46.8′	135°17.2′	u	2.3	u
	246	57°58.6′	136°16.3′	u	u	u
	421	58°47.1′	136°24.7′	u	u	u
	358	58°13.8′	135°23.7′	—	3.1	u
	440	59°00.1′	135°13.0′	u	1.6	u
	443	58°13.2′	134°38.1′	u	u	u

[a] Symbols: — = sample not analyzed; u = undetectable.

TABLE 2. *Presence in surface water of factors inhibitory to the biotin mutant of Serratia marinorubra*

Station no.	Latitude N	Longitude W		Sample[a] (pg/ml)	Internal standard[b] (pg/ml)	Recovery (pg/ml)
257	58°44.4′	136°18.0′	Treated	1.6	12.8	11.2
			Untreated	0.3	11.6	11.3
247	57°57.3′	136°22.6′	Treated	1.4	12.8	11.4
			Untreated	u	9.6	9.6
233	57°49.7′	135°24.8′	Treated	1.0	12.0	11.0
			Untreated	0.2	11.0	10.8

[a] u = undetectable.
[b] Internal standards were given 10 pg of biotin per ml.

in a few surface waters. This factor could be removed by acid-petroleum-ether extraction by use of the method of Litchfield and Hood (6). Three samples treated with petroleum-ether extraction showed (Table 2) a higher range of concentrations than the untreated samples. All the seawater samples for biotin and niacin bioassay were analyzed after petroleum-ether extraction. The reported values can thus be assumed to be the optimal concentrations one can expect to obtain by the technique employed during this investigation. Carlucci and Silbernagel (4) also reported inhibitory effects of different seawater samples on *Serratia* mutants. The biotin mutant used by the above authors was found to be not inhibited by deep water samples from coastal and central north Pacific.

The range of biotin concentrations found in southeast Alaskan waters is compatible with results obtained by Carlucci (Abstr. 11th Pacific Science Congr., Tokyo, vol. 2, p. 33, 1966), who reported 0.65 to 3.2 ng/liter in the north Pacific Ocean. Antia (1), using an unidentified bacterium, found that there was no detectable biotin (1 ng/liter) in two samples of water collected at a station 43°N, 141°W from depths of 5 and 3,000 meters. Carlucci and Belser (Bacteriol. Proc., p. 3, 1963), on the other hand, found a high concentration (10 μg/liter) of biotin in water off the pier at Scripps Institution of Oceanography. Litchfield and Hood (6), using the same *Serratia* mutants used in this investigation, reported that, of a total of 44 samples from the Gulf of Mexico and adjacent bays, only one location showed the presence of biotin definitely. Belser (2,) also using *Serratia* mutants, found that of a total of 29 samples of seawater from one area 16 (55%) showed detectable quantities of biotin.

There has been only one investigation with regard to the distribution of niacin, which is that of Belser (3), who found that 50% of near-shore samples contained detectable quantities of niacin.

Natarajan and Dugdale (7) discussed the importance of thiamine in the marine environment, and found that in coastal waters the concentrations tend to be very high, up to 490 ng/liter. During this study, the combined bioassay of samples from the same region for three vitamins tends to agree with the conclusion that, with reference to this location, thiamine is the important vitamin. Although biotin is found in a few locations, the range of concentration is many orders of magnitude less than that found for thiamine. Biotin and thiamine are found together only in one location (station 358).

The random discontinuity in the distribution of the three vitamins, and the scarcity of other available biological data for this region, makes any positive conclusion speculative. The measurement of instantaneous distribution of any growth factor may also be unrewarding, and sometimes misleading. The data collected so far in this region show (7) that thiamine may act as a limiting nutrient or may influence the species composition, or may have both effects. Results of experiments (Natarajan, *unpublished data*) with surface water samples, in which the uptake of ^{14}C-labeled thiamine is measured concurrently with the photosynthetic ^{14}C-uptake, indicate a high correlation (correlation coefficient of 0.96), showing the importance of thiamine auxotrophs in the productivity of this area. A similar relationship was also seen in water from 10- and 25-meter depths. Although there are indications that thiamine plays an important part in the productivity of this area, the possible implications of other growth factors should not be neglected.

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